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On the Genetic Character of Neoplastic Cells as Determined in Transplantation Experiments

With Notes on the Somatic Mutation Theory*

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There are several studies concerned with the elucidation of the genetics of transplantability of neoplasms in mice (12, 33). The results have varied with the inbred stocks used, but one fundamental question is raised by all: Is the transplantation pattern of neoplastic cells different from that of normal cells?

The earliest investigations on transplantation of neoplastic and normal tissues were made with genetically heterogeneous animals and did not permit a clear-cut answer to this question. Strong (52) and Little and Strong were the first to study the fate of grafted tumors in closely inbred lines of mice that were genetically homogeneous. They found two adenocarcinomas of the mammary gland originating in their dBr stock, which could be transplanted within the line of origin and to first generation hybrids but failed to grow in another stock (Bagg albino).

Bittner (4) found two tumors originating in F_1 hybrids derived from Little's dBr stock and Bagg albino. These were transmissible to almost all F_1 animals, but failed to grow in the parental stocks. In postulating a genetic basis for these findings, Bittner assumed that the simultaneous presence of 7 or 8 genes was required for transmission of tumors originating in the F_1 hybrids. Similar observations were made by MacDowell and Richter with leukemias arising in their inbred stock C58. Kirschbaum and Strong (27) have confirmed the transplantation pattern found by Bittner with leukemias arising in hybrids of their inbred stocks F and CBA. They point out that their results are consistent with those found for transplantation of normal spleen by Bittner (6). Thus, Strong reversed the conclusion reached in his former study (53), which brought experimental evidence for the theory that "the tumor tissue has deviated from the definitive somatic cell that gave rise to it by a process analogous to genetic mutation." This conclusion was reached by Strong on the basis of observations indicating that the genetic constitution of different neoplasms arising spontaneously in the same host may differ.

Different results were obtained by Schweitzer and Furth. In transplantation studies with leukemic cells derived from mice of the inbred high leukemia stock Ak and low leukemia stock Rf and their hybrids, they observed that spontaneous leukemias originating in the high leukemia stock Ak and the low leukemia stock Rf are readily transplantable to stocks of origin but not to mice of the opposite stock. Both are transplantable to first generation hybrids. They also found that leukemias arising in the F_1 and other hybrids behave in transplantation experiments like those arising in the leukemia stock Ak; that is, they are transplantable to the Ak parental stock and hybrids, but not to the Rf stock. Thus, under the conditions of these experiments, the transplantability of neoplastic cells in F_1 hybrids differs from that of normal F_1 cells, as reported by Bittner (6) and Little and Johnson.

These studies were extended by Furth and Barnes to the transplantation relations of cells from leukemias induced in the Ak and Rf stocks. They have shown that the transplantation patterns of neoplastic blood cells from several leukemias induced in the same inbred stock may differ among themselves and from spontaneous leukemias. Leukemias arising in the F_1 and F_2 hybrids, for example, could be grafted to mice of both parental stocks or to neither of them, though they were readily transplantable to hybrids; or they could be passed to only one of the parental stocks.

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These differences were interpreted as supporting the hypothesis that the cells of the induced leukemias are genetic mutants.

These studies of Furth and Barnes do not contradict those of Schweitzer and Furth, as Gorer (19) maintains, but indicate a conspicuous difference in the behavior of malignant cells from induced and spontaneous leukemias. However, because of the apparent contradictions resulting mainly from the use of different inbred stocks of mice by different investigators, it seemed desirable to complement the studies mentioned above by a determination of (a) the genetic relation of normal Ak and Rf cells in transplantation experiments, (b) the transplantation relationship of spontaneous and induced neoplasms other than leukemias, and (c) the effect of the substitution in similar genetic studies of another low leukemia stock for the low leukemia stock Rf.

The first of these problems has already been answered in a preliminary report of Kaliss and Robertson. They found that normal spleen from Ak and Rf mice can be transplanted within the line of origin and to F_1 hybrids, but normal spleens of F_1 mice will not grow in the parental lines, though they can be grafted on related F_1 mice. Thus a *normal* tissue (spleen) from Ak and Rf mice and their first generation hybrids behaves in transplantation experiments as normal tissue from stocks studied by other workers.

Investigation of the second problem, that is, transplantation relationships of spontaneous and induced neoplasms other than leukemias began several years ago. As the work progressed the task appeared greater

TABLE I: TRANSPLANTABILITY OF TUMORS ARISING IN STOCKS AK AND RF AND THEIR F_1, F_2, AND F_3 HYBRIDS

Tumor		Recipient mice									
		Ak		Ak fostered by C3H		Rf		F_1 (Ak×Rf)		F_2	
No.	Type	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+
ARISING IN AK MICE											
Akh 678	Breast tumor	27/28	97	18/19	95	0/23	0	15/15	100		
Ak 817	Induced sarcoma	4/37	11			17/20	85	61/71	86		
Ak 835	" "	43/45	96			0/14	0	19/29	66	17/24	72
Ak 836	" "	1/5	20								
ARISING IN AK FOSTERED BY C3H											
F. Akh 599	Breast tumor	33/33	100	4/4	100	0/4	0	4/6	67		
F. Akh 600	" "	38/44	86	4/4	100	0/5	0	23/26	88	0/5	0
F. Akh 601	" "	17/17	100	9/9	100	0/7	0	3/4	75		
ARISING IN RF MICE											
Rfb 718	Angioendothelioma	0/22	0	0/6	0	82/87	94	13/15	87		
Rf 961	Induced sarcoma	8/13	62	2/9	22	41/51	80	7/11	64	4/11	36
ARISING IN F_1 MICE											
Ma 45	Induced sarcoma							2/17	12		
Ma 145	" "							2/15	13	0/6	0
Ma 148	" "							2/15	13	0/6	0
Ma 707	" "	0/10	0	0/10	0	0/18	0	23/26	88	4/13	31
M 768	" "	0/16	0	0/11	0	0/34	0	20/63	32	2/15	13
ARISING IN F_2 MICE											
Ma 387	Adenoma of lung	10/11	91	11/12	92	0/13	0	12/16	75	10/14	71
Hb 183	Carcinoma of skin	6/28	21			2/38	5	38/62	61	11/29	38
Ma 92	Induced sarcoma									1/7	14
Ma 104	" "	17/19	89	7/10	70	0/16	0	33/46	72	14/34	41
Ma 508	" "							0/6	0	1/3	33
M 788	" "	5/15	33			1/27	4	33/62	53	8/36	22
ARISING IN F_3 MICE											
Ma 40	Induced sarcoma	0/6	0			0/7	0	4/15	27	0/7	0
ARISING IN F_2 BACKCROSS TO AK											
Ha 202	Splenic reticulum cell sarcoma	36/39	92	7/13	54	0/29	0	46/68	68		

The fractional numbers indicate the number of animals tested (denominator) and the number of takes (numerator). All tumors are spontaneous unless otherwise indicated.

than anticipated, and at the onset of the present war the data accumulated were already bulky, yet not wholly conclusive. Nevertheless, it became desirable to bring this type of expensive and time-consuming research to a temporary halt and release the data of interest.

Transplantability of tumors arising in stocks Ak and Rf and in their hybrids.—Spontaneous tumors are rare in both Ak and Rf stocks, and the majority of data accumulated are concerned with sarcomas induced by methylcholanthrene and breast tumors induced by the milk factor. It is noteworthy that the genetic studies of Bittner (4) referred to were made with breast tumors, which at that time were regarded as spontaneous growths. The results of our experiments are summarized in Table I.

The transplantation behavior of the spontaneous mammary carcinoma Akh 678 and of spontaneous angioendothelioma Rfb 718, arising in the inbred stocks Ak and Rf respectively, was indistinguishable from that of normal tissues. These tumors grew well in animals of the stocks of origin and in their F_1 hybrids. Of the 3 spontaneous tumors arising in hybrids, one, Ma 387 (F_2), behaved like an Ak neoplasm, while another, Hb 183 (F_2), resembled normal tissue in that it was readily transmissible to other hybrids but only poorly to the parental stocks. A third spontaneous neoplasm (Ha 202) could be grafted with greater success on the ancestral Ak mice than on hybrids, and not at all on the ancestral Rf mice. The 3 mammary tumors arising in Ak mice that had been fostered by C3H mice behaved like the breast tumor that arose in the nonfostered Ak mouse.

Of the tumors induced in the inbred stocks Ak and Rf, two (Ak 817 and Rf 961) went in both inbred stocks and in the F_1 hybrids. The Ak mice were the poorest hosts for the Ak tumor, but the Rf tumor could be grafted about equally well in all these stocks. Induced sarcoma Ak 835, like the breast tumor Akh 678, could be readily transmitted to the parental Ak stock, but not at all to the Rf stock. One induced sarcoma, Ak 836, was insufficiently tested to draw any definite conclusions, but it is noteworthy that it took in only 1 of 5 Ak mice tested, while transmission of spontaneous leukemias seldom if ever fails in this stock.

Of the tumors induced in hybrids, the F_1 tumors tested behaved like normal tissues in that they did not grow in the parental stocks but did grow in the hybrids. Of the F_2 tumors, one (Ma 104) behaved like an Ak neoplasm, while M 788 grew poorly in one parental stock, moderately well in the other, and best in the F_1 hybrids. The F_3 tumor (Ma 40) grew not at all in the parental mice, but grew poorly in F_1 mice.

Genetic relationship of Ak and C3H stocks as indicated by transplantation experiments.—The C3H mice of the Jackson Memorial Laboratory, famous for the high incidence of mammary cancer and for a strong "milk factor," have a low incidence of leukemia. For this reason they seemed particularly useful in studies on the possible existence of a "milk factor" influencing the occurrence of leukemia. Accordingly, reciprocal crosses were made between C3H and Ak mice (14), and the results were as follows:

	Spontaneous Leukemia		
	Male, per cent	Female, per cent	Male and female, per cent
Ak stock	61	53	58.0
C3H stock			0.3
Ak female × C3H male	48	54	50.0
C3H female × Ak male	39	28	34.0

The results suggest the existence of a nursing influence and corroborate previous findings (6) on reciprocal crossing between the high leukemia stock Ak and the low leukemia stock Rf:

	Spontaneous Leukemia		
	Male, per cent	Female, per cent	Male and female, per cent
Ak stock	71	67	69
Rf stock	2	1	2
Ak female × Rf male	16	28	22
Rf female × Ak male	15	9	12

These figures indicate that the low leukemia Rf mice, unlike the low leukemia C3H mice, definitely inhibit the spontaneous development of leukemia in F_1 hybrids.

Since our conclusions on differences in transplantability between spontaneous and induced leukemias were based on observations on Ak and Rf mice and their hybrids, it seemed desirable to investigate the transplantation relationship between Ak and C3H mice. The results were wholly unexpected. The figures in Table II show that the C3H mice, unlike the Rf mice, are susceptible to grafts of Ak leukemic cells and that the leukemias of first generation hybrids can be readily transmitted to both parental stocks and to first generation hybrids. Here is another example indicating a difference in susceptibility to spontaneous and transmitted leukemia.

Numerous tests were made to find out if foster nursing had any influence on the susceptibility to transmitted leukemias, as described by Law. In the few experiments in which a resistant foster mother (Rf) was used, the nurslings were about as susceptible as mice of the same stock nursed by their mothers.

Table III shows the results of transplantation experiments with tumors. All but one of the tumors tested in this group were spontaneous breast carcinomas. The induced tumor (Ak 817) was a sarcoma. Three breast carcinomas arose in Ak mice that were fostered by C3H

mice; the fourth was spontaneous. All four Ak tumors took in both inbred stocks, though much better in the stock of origin, and well in the hybrids. There is no clear cut evidence that C3H animals fostered by Ak are less antagonistic hosts than unfostered C3H mice. Tumors arising in the C3H stock grew well in the stock of origin. Two (H 91, H 31) grew poorly in the Ak stock. Tumor Hx 16 did not take in Ak mice, but the number of mice tested was small. There is no indication that Ak mice fostered by C3H are more susceptible recipients than nonfostered Ak mice. The F_1 tumors grew equally well in both parental stocks and well in the hybrids.

Thus transplantation experiments indicate some genetic relation between the albino Ak and the agouti C3H mice, even though the incidence and types of neoplasms occurring spontaneously in these stocks are entirely different.

TABLE II: TRANSPLANTATION EXPERIMENTS WITH LEUKEMIAS ARISING IN THE AK STOCK AND THE F_1 AND F_2 HYBRIDS BETWEEN AK AND C3H

Leukemia		Recipient mice											
		Ak		Ak fostered by C3H		C3H		C3H fostered by Ak		C3H fostered by Rf		F_1 (Ak×C3H)	
No.	Type	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+
ARISING IN AK MICE													
*Akf 5	Spontaneous leukemia	17/17	100	20/20	100	3/5	60	9/14	64			38/38	100
*Akh 106	" "	8/8	100	8/8	100	9/11	82	2/2	100	5/5	100	15/15	100
Ak 946	Induced leukemia	38/45	84	19/19	100	4/4	100			4/4	100		
Ak 966	" "	15/25	60			2/2	100	2/5	40				
Ak 970	" "	59/63	94			13/20	65			3/7	43		
Ak 1030	" "	19/20	95	7/7	100	1/5	20	1/3	33				
ARISING IN F_1 (AK×C3H) MICE													
KH 201	Spontaneous leukemia	25/26	96	0/4	0	12/13	92					23/23	100
KH 157	" "	4/15	27			4/17	24					24/25	96
HK 117	" "	16/22	73	12/12	100	8/9	89	5/7	71	2/4	50	13/13	100
HK 23	" "	27/37	73	0/6	0	7/18	39	0/4	0			27/30	90
ARISING IN F_2 MICE													
Bb 12	Spontaneous leukemia	7/7	100	8/8	100	13/14	93	2/2	100			5/5	100

* Cf. (50) for previous experiments with these leukemias.

TABLE III: TRANSPLANTATION EXPERIMENTS WITH TUMORS ARISING IN AK AND C3H STOCKS AND IN THEIR HYBRIDS

Tumor		Recipient mice									
		Ak		Ak fostered by C3H		C3H		C3H fostered by Ak		F_1	
No.	Type	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+
TUMORS IN AK MICE											
Akh 678	Breast tumor	27/28	96	18/19	95	8/20	40	19/21	90	5/5	100
Ak 817	Induced sarcoma	4/37	11			0/15	0			2/5	40
AK FOSTERED BY C3H											
F. Akh 599	Breast tumor	33/33	100	4/4	100	9/20	45			10/10	100
F. Akh 600	" "	38/44	86	4/4	100	7/28	25	7/8	88	7/9	78
F. Akh 601	" "	17/17	100	9/9	100	6/17	35			14/15	93
TUMORS IN C3H MICE											
H 31	Breast tumor	2/26	8			18/19	95	1/1	100	27/28	96
Hx 16	" "	0/4	0	0/17	0	5/5	100	10/10	100	20/20	100
H 91	" "	13/43	30	15/26	58	41/41	100	15/15	100	29/29	100
TUMORS IN F_1 (C3H×AK)											
HK 53	Breast tumor	2/13	15	1/9	11	4/15	27			29/31	94
HK 60	" "	4/9	44	0/10	0	14/20	70			13/13	100
HK 63	" "	12/15	80	6/8	75	8/9	89	4/4	100	18/18	100

DISCUSSION

With the accumulation of experimental data it has become evident that observations on the genetic behavior of neoplasms made with one stock of animals may not be applicable to other stocks and that the pattern of inheritance for a given type of neoplasm, *e.g.,* leukemia, may vary among different inbred stocks. Yet these considerations are often omitted, and valuable contributions that are complementary are cited as conflicting.

Gorer (19), in a recent study on the immunological basis of the acquired immunity to transplantable leukemia, cites our observations on inherited resistance as conflicting with his. None of our studies concern acquired resistance, which depends on immunological difference between host and grafted cells, as Gorer (18), more recently Eisen and Woglom, and others have shown. Gorer finds it hard to explain why a neoplasm occurring in only 2 per cent of mice of an inbred stock (Rf) should be transplantable to almost every member of this stock. Factors responsible for the production of a tumor are not identical with those governing its transplantability. While the incidence of spontaneous neoplasms varies in inbred stocks of mice and may be very low, it is rather the rule than the exception that members of such stocks are uniformly susceptible to any neoplasm occurring in the stock. The incidence of spontaneous neoplasms can be further reduced by hybridization; yet first generation hybrids are, as a rule, as susceptible to tumor grafts as mice of the parental stock.

When it was discovered that the malignant cells from spontaneous leukemias arising in hybrids between mice of a high leukemia stock (Ak) and of a low leukemia stock (Rf) genetically resemble cells of the high leukemia ancestral stock, it became desirable to study the transplantation behavior of induced neoplasms. It was found that these may differ from normal host cells and from spontaneous neoplasms and may also differ among themselves (14). The two sets of data are not conflicting, as Gorer believes. These data were not in harmony with the thesis based on observations of others, *i.e.,* that neoplastic cells of F_1 hybrids behaved invariably like normal cells in transplantation experiments in that they required for successful grafting the simultaneous presence in the host of factors contributed by each parent. It seemed desirable, therefore, to test whether this thesis held also for normal cells of the stocks studied by us. The experiments thus far performed by Kaliss and Robertson indicate that normal splenic fragments from F_1 animals (Ak × Rf hybrids) could be grafted to F_1 hybrids but not to Ak and Rf mice, while splenic grafts from animals of the Rf and Ak stocks grew in the stocks of origin and their F_1 hybrids, but not in the unrelated inbred stocks. Thus normal tissue in these stocks behaves in the same manner as normal tissue of other stocks described, and it can now be stated with certainty that spontaneous leukemia cells of F_1 hybrids of the stocks studied differ in transplantability from normal F_1 cells. The experiments described in this report show that induced sarcoma cells, too, may differ from normal cells of the host.

It was fortunate that the stocks Ak and Rf happened to be used, for this relationship cannot be demonstrated with all inbred stocks of mice. Kirschbaum and Strong (26), for example, in extensive studies on the transplantability of leukemia cells in F_1 hybrids, found in the stocks used by them no significant difference between normal cells and leukemia cells. Experiments were performed to find out if in the genetic studies substitution of the low leukemia stock Rf with another low leukemia stock would alter the results. The well known C3H stock of Little and Bittner was chosen because the incidence of leukemia in this stock is well below 1 per cent, while that in Rf mice is 2 per cent. Both Ak and Rf mice are inbred albino lines, while the C3H mice are agouti. The incidence of spontaneous leukemia was not significantly lowered in C3H × Ak hybrids as it is in Rf × Ak hybrids. The transplantation experiments here described indicate that the low leukemia stock C3H mice are susceptible to neoplasms occurring in Ak mice, to which the low leukemia stock Rf animals are resistant. This is another example showing that inbred stocks may be susceptible to transplantation of leukemic cells, even though this disease is almost nonexistent in them.

With inbred stocks uniform susceptibility or resistance is expected, but this is not true for heterotransfers among Ak and C3H mice, the heterotransfers being successful but not universally so, while homologous grafts take in almost 100 per cent of the mice. If the Ak and C3H stocks are genetically homogeneous, this observation with C3H mice cannot be explained on a purely genetic basis.

The somatic mutation hypothesis.—Can our transmission experiments be cited as supporting the hypothesis that mutations in somatic cells are the immediate causes of some neoplasms?

Boveri is credited with the theory that cancer is due to chromosomal changes, although he himself traces the idea to von Hansemann.[1] This view has since been propounded by numerous workers, with but slight modification, without convincing evidence having been presented for its support. Observations made by Bittner; Cloudman; Little and Strong; and Tyzzer

[1] The historical background of this theory is well presented by K. H. Bauer in his "Mutationstheorie der Geschwulstenstehung" (Berlin, Springer, 1928) and the supporting and contradicting views available at that time are ably discussed.

(33) have shown that different tumors arising spontaneously in genetically homogeneous (inbred) mouse stocks or even in the same animal behaved differently as concerns their growth rate and percentage of takes when transplanted to hybrids. These differences were explained by assuming genic differences between the tumors. The observation (Bittner, Cloudman, Strong) that with repeated transfers the number of takes in hybrids increased need not be regarded as contradicting the hypothesis. Little (33, p. 290) says, "In every case the change has been in the direction of decreased specificity, and there have been ratios indicative of fewer factors after the change than before it. Since the changes appear to be sudden, and since they are perpetuated from one cell generation to another, they are properly definable as mutations. It will, of course, be necessary to discover a method of identifying the genes borne within the tumor cells before the mutations can be considered as established "gene" mutations. They are, however, abrupt genetic modifications which are self-perpetuating."

The hypothesis that tumors are initiated by somatic mutations is founded on the assumption that transplantation differences are based upon genic differences. There are, however, numerous investigations along different lines that have been likewise interpreted as supporting the mutation theory of cancer (Curtis, Dunning, and Bullock; Haddow and Robinson; Henshaw; Koller; Lockhart-Mummery; Mackenzie and Muller; and others.) Some of these studies will be reviewed elsewhere (13).

Attempts at demonstrating chromosomal changes associated with neoplasms (reviewed by Kirschbaum and Strong; Levine; Ludford; and more recently by Berrill; and Biesele, Poyner, and Painter) have produced some supporting evidence. The reported alterations deal with changes in chromosome number, size, shape, and deviations from a normal mitotic picture. Some of these will be reviewed elsewhere (13). Work of this type encounters the technical difficulties associated with mammalian cytology, and the dilemma whether the functional physiological aberrations affecting the neoplasms lead to mitotic abnormalities or whether an initial upset in the mitotic mechanism leads to a neoplastic growth. An intracellular virus, among other possible factors, may conceivably produce mitotic aberrations, and the virus may be a silent, inseparable companion of the tumor cells.

Loeb was at first a proponent of the mutation hypothesis (37), but later became one of its opponents. He states (38, p. 177) "(1) That the individuality differential of the cancer cells is the same, or almost the same, as that of the normal cells from which they originated, (2) that changes in growth energy and transplantability of tumor cells may take place independently of genetic changes, (3) that the development of tumors may vary greatly in individuals possessing the same, or almost the same, individuality differential, and (4) that what is constant is the proportion of tumors which develop in a certain strain." Somatic mutation, "as a rule, should affect only a single cell, and it would be a very remarkable coincidence if the same type of mutation would simultaneously occur in all the cells composing a typical tissue unit."

The first of these objections does not hold for the observations here reported, as indicated by the varied behavior of the induced leukemias in contrast to the uniform behavior of normal lymphoid cells and the likewise uniform, though somewhat different, behavior of cells from spontaneous leukemia.

Changes in growth energy, more precisely a shortening of the course of illness, are the rule in our observations. Changes in transplantability, more precisely a decrease in specificity, have not been seen by us since we have been working with highly inbred stocks. This phenomenon has been a favorite subject of earlier discussions. Bittner (5) says that "With transplantable tumors, a mutation is easily discernible due to an increase in the proportion of susceptible individuals in the various generations which show segregation of the susceptibility factors. This difference is due to the loss of one or more of the factors necessary for progressive growth. Mutations in the reverse directions have not been recorded."

If mutations are the basis for this phenomenon, it should not be regarded as unusual to find it unidirectional, *i.e.,* leading to a decrease and never to an increase in specificity. The usual transmission experiments are suitable to demonstrate readily an increase but not a decrease in virulence, since the more rapidly multiplying cells outgrow the less "virulent" ones.

The last argument of Loeb cited is not supported by our observations. The neoplastic change is often multicentric but does not affect simultaneously all cells composing a typical tissue. The transmission experiments with small numbers of counted leukemic cells (17) can be cited in this connection and the well known observation that individuals may remain free from neoplasm permanently or over long periods of time following removal or destruction of a malignant growth.

Rous and Kidd, opposing the mutation theory of cancer, state: "All of the mutated cells which have thus far come to attention continue to obey the general laws of organization, and they do not proliferate at the general expense; in a word, they do not form tumors. The somatic change may find expression in colored dots on a corolla, made up of a mosaic of cells differing from the rest only in their unusual color; or a

spray of delphinium may have white flowers while all the rest of the plant is blue; or a fern frond possess one ruffled pinna amongst a generality which are smooth. A guinea pig may have a patch of skin of a hue unwarranted by anything in its lineage, an obvious somatic mutation, which dies with the animal. Corolla dots, delphinium flowers, fern pinna, and skin patch they all remain, none constituting a tumor."

J. S. Haldane questions the view of J. L. Smith favoring the theory that the growth of a malignant tumor has lost its coordinated character owing to an injury to chromosomes, this injury being admitted as a mutation from cell to succeeding cells. He admits that the idea is well founded that chromosomal injury may alter the character of the descendants of the injured cell. But the known chromosomal changes, he believes, are of less fundamental kind than would be required to explain malignancy. Moreover, coordinated growth is retained by cells with chromosomal changes.

If the emphasis in the definition of somatic mutation is placed not on colors, sizes, textures, magnitude of alteration, etc., but on characters of any kind acquired abruptly, discontinuously, and retained permanently by a given cell lineage, it should include the neoplastic change.

Mutations are believed to be relatively rare occurrence, while cancer is common. On the one hand, this statement is incorrect; the mutation rate in x-rayed drosophila may be as high as 15.6 per cent (54). On the other hand, the frequency of neoplastic change is more apparent than real. Conditions in the mammalian body are extremely sensitive for the disclosure of a cancer cell. This sensitivity finds no parallel in the usual material of the geneticist. The number of normal lymphocytes in the mammalian body amounts to billions, but if one assumes malignant properties the organism will register it. Considering the tremendous number of cells used in transmission experiments, the constancy of the characteristics acquired when the cells turn malignant is more impressive than their variability. Recent studies (15) concerning the onset of both induced and spontaneous leukemia made by means of bioassays indicate that the onset of this malignant change is rather sudden. It may be preceded by a succession of histological changes not yet sufficiently studied, but the malignant change as indicated in transmission experiments is fairly abrupt. The constancy of newly acquired characteristics of neoplastic cells of different lines kept under identical conditions has been well demonstrated by Warren H. Lewis by means of tissue cultures.

Loeb (38) and Rous and Kidd, on the contrary, emphasize that cancers are the outcome of successive cell changes, while a succession of cell mutations of the recognized kind is rare, if it occurs at all. But it is admitted with no hesitation that viruses often undergo successive alterations (Kunkel, Andrewes), and such is postulated in the course of carcinogenesis due to viruses. Why deny potentialities of variability to mammalian cells and admit it to viruses?

The idea that cancer cells are the consequence of successive cell changes is not a proved rule. There are numerous impressive examples to the contrary, *e.g.*, cells of hypernephromas, and of several other benign and malignant tumors having their family resemblance, are often sharply circumscribed in their primary sites with no transitional forms to normal cells. The common pulmonary adenomas of mice originate in flat alveolar lining cells; these assume a cuboidal or cylindrical form and proliferate, producing a benign growth with similar gross and microscopic characteristics.

Subthreshold neoplastic states are common with certain types of neoplasms and have been adequately considered by Rous and his associates. In a disorganized tissue with many atypical cell forms it is difficult to identify a neoplastic cell, if it is possible at all. Morphological studies may be as deceptive in the demonstration of step-like changes in the origin of cancer as they are in the histogenesis and interrelationship of different cell types.

The multiplicity of neoplasms of different kinds occurring in the same individual either spontaneously or as a response to methylcholanthrene or 2-acetaminofluorene (55) or other stimuli offers difficulties of explanation to both the virus and the mutation theory. The former would imply the simultaneous activity of 10 or more viruses in the same individual and the latter the simultaneous occurrence of as many mutations. Both are conceivable, but in some instances the theory of hereditary dysplasia is a more plausible explanation.[2]

The virus theory implies that man and animals are carriers of numerous viruses and that they are so changed in response to altered conditions as to work on the cells with which they are associated, with the result that these become tumor cells. A pathogenic variant of the virus arises that "would not be trans-

[2] A case of remarkable multiplicity of neoplasia studied recently by one of us is a good example. It concerns a woman 54 years of age who had, in addition to an enormous myxofibrolipoma in the mediastinum, which brought about her death, the following neoplasms: (a) malignant: carcinoma of liver (in the absence of cirrhosis) with metastases to regional nodes; carcinoma of thyroid with metastasis to regional node; (b) benign: lipomas of kidneys, multiple, in part symmetrical; papillary adenoma of kidney, fibromas of kidney (numerous); leiomyomas of uterus; adenomas of thyroid; lipomas of subcutaneous tissue; nevi of skin; hemangioma of bone; and a few debatable tumors. The lipomas of the kidney deserve further comment. They occupied parts of the kidney, as if they were normal components of it. There was no evidence of either destruction, compression, or invasion of renal parenchyma by the neoplasm.

mitted to other animals under natural circumstances for reasons as yet obscure but would be a 'dead-end' virus, not concerned in the production of other neoplasms, though the harmless source virus liable to the same or other variations would be passed on." (47)

It is a fact that viruses may actuate tumors and be responsible for the maintenance of some tumor cells, but an explanation of all cancers on the basis of the virus theory meets obstacles as complex as those opposing the theory that mutation is the sole cause of cancer. If there exist numerous tumor viruses in the normal body, where do they reside? The tumor viruses are conceived as intracellular parasites. Shall we suppose that different normal cells carry different viruses waiting for opportunities to change them into cancer cells?

With the discovery of the milk factor the trend to regard viruses as the nearest causes of many cancers is steadily mounting (47). The milk factor, unlike the Rous virus of chicken sarcoma, multiplies in normal animals and is handed down to successive generations of hosts without producing a neoplastic change. It may reside in tumor cells, but there is no evidence that it is responsible for the maintenance of the tumor cell. Some viruses, as those of papillomas, resemble those of communicable diseases. Virus is recovered in abundance from chicken sarcoma but not from carcinoma produced by the papilloma virus. The tumor viruses are heterogeneous, and there is still much to be learned about each, particularly as concerns their normal habitat and relation to the cancer cell. The suggestion that they are endogenous should not be overlooked, and if so they may result from cell mutations.

The theory of endogenous origin of the active principle of chicken sarcoma and the experiments supporting this theory have been reviewed by Murphy (45), its chief proponent. The difficulty in regarding agents of chicken tumors as similar to those producing infectious diseases "comes when one attempts to expand this conception to cover the varied types of tumors, each with an agent, not only limited in its action to a species and to definite cell groups in the organism, but with the property of causing them to multiply and differentiate into various complex tissue types. For the chicken tumor group alone we would have to consider a number of varieties of such viruses, and if the idea is accepted for other species, to explain fixed individualities, histological and biological, we would have to imagine almost a separate variety of virus for each tumor. In fact the chicken tumor group may be considered as giving evidence against the parasite theory" (45).

Loeb (37) was the first to draw the parallel between organizer phenomena and the filterable agents of chicken sarcoma. Murphy (44) has extended this idea, assuming that agents of chicken sarcoma act by producing a mutation and named them "transmissible mutagens." If carcinogenesis is looked upon in the light of abnormal differentiation, it is superfluous to assume that it is accompanied by mutations (J. B. S. Haldane). It is a fundamental theorem of developmental mechanics that all somatic cells of an organism have the same chromosomal makeup, and differentiation is not accompanied by genic changes (cf. Medawar). Carcinogenesis by chemical evocators is readily explained on the basis of this theory provided the neoplastic change is not accompanied by chromosomal alterations. Cancer may be regarded as an escape from the organism's individuation field with consequent proliferation over and above normal (cf. Needham). But if this theory is applied to virus tumors the additional assumption is needed that these evocators produce evocators of their kind. This has actually been postulated by Needham, who compares carcinogenesis by virus with neural plate induction. The latter could also be propagated indefinitely by cell-free extracts of them. The synthesis of further supplies of evocators of neoplasms may be stimulated autocatalytically like trypsin from trypsinogen or plant viruses in their hosts. (46, p. 268).

Abnormal cells may contain abnormal components other than exogenous viruses that may be antigenic in their hosts and be responsible for immunological phenomena simulating those occurring in virus infections.

The suggestion that tumor viruses act by producing mutations, recently considered again by Jones, has met with serious objections. To accept this sequence of events one would have to suppose not only that neoplastic viruses produce mutations wherever they act (Ludford), but also that the mutations produced are of a peculiar type, depending on the character of the virus, *e.g.,* osteochondrosarcoma (Rous).

The theory of somatic mutation here advocated for certain neoplasms cannot be proved without direct evidence for a chromosomal change. It may be strengthened greatly by more conclusive indirect evidence, such as the line of investigation proposed by Mottram. If the tumors that he produced by irradiating a precancerous lesion were due to mutations, then the number produced should, according to the researches of Timoféeff-Ressovsky, be related to the dose applied, but not to the dosage rate; whereas if they were due to a cause other than mutation, both dosage and dosage rate should affect the number. Mottram states that he had begun such a test by giving to mice painted with benzpyrene for 70 days 90 r gamma radiation, in one case in a week, in another case in 5 hours, and in another case in 1 hour. The outcome of that experiment is awaited with much interest. Similar studies

are possible with respect to the induction of neoplasms by ultraviolet light (cf. Mackenzie and Muller).

The very recently reported fundamental observations of Earle are best explained in the light of the mutation theory. Normal cells kept in tissue culture over long periods of time underwent a gradual morphological change, assuming the characteristics of malignant cells, and produced sarcoma when injected into mice. Why should the general biological properties of variability, ability to differentiate normally or abnormally and to mutate be denied to living cells of mammals? If one normal cell assumed the character of a malignant cell, it would gradually outgrow all normal cells. Nevertheless, to accept the idea that some cancers are mutations evidence is wanted that their origin is accompanied by chromosomal change. At the present time the strongest indication for such changes are the transmission experiments here discussed.

SUMMARY

1. The transplantation pattern of neoplastic cells arising in the same pure stock mice and their known hybrids varies greatly, while that of a normal tissue thus far studied follows a single pattern. Tumors *induced* in 2 different inbred lines (Ak and Rf) take well in their hybrids, and they may take in both inbred lines, or only in the line of origin. Tumors induced in the hybrids take well in hybrids; they may take well or poorly in one or both of the parental lines, or not at all in either parental line.

2. Tumors arising *spontaneously* in members of the inbred Ak or Rf line are transplantable to other animals of the same line, and to the F_1 hybrids, but they are not transplantable to the unrelated line. Neoplasms arising in hybrids from these two inbred lines go well in hybrids, though not always. They may go well in one pure strain, but not in the other, or go poorly in both.

3. Both spontaneous and induced leukemias arising in the high leukemia albino stock Ak take well in the stock of origin and fairly well in the inbred unrelated agouti stock, C3H, in which leukemia is extremely rare. Leukemias arising in the F_1 hybrids between these two stocks take well in F_1 hybrids, and may take well or fairly well in both parental stocks. The same relation exists between Ak and C3H mice with regard to transplantability of neoplasms other than leukemia.

CONCLUSIONS

The transplantation pattern of normal tissues of inbred mice thus far studied is monotonously uniform in mice of pure lines and in known hybrids. The transplantation pattern of leukemias and tumors arising in the same inbred stocks may differ greatly from that of normal tissue. Induced leukemias and tumors may differ greatly among themselves, but there is a transplantation pattern characteristic of each neoplasm, and this is retained through numerous passages.

If differences in transplantation pattern are based on genic differences, then this abrupt, discontinuous, and irreversible change is not an abnormal differentiation but a somatic mutation. The causes of cancers are complex and viruses doubtless are prominent among them, but the immediate change that makes some cells cancerous in the course of chemical carcinogenesis may be a somatic mutation.

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A Genetic Analysis of the Induction of Tumors by Methylcholanthrene

VI. Epidermoid Carcinomas and Associated Tumors in Mice of the F_4-F_7 Generations of the NH Descent

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Several reports in the present series on the results obtained by the subcutaneous injection of methylcholanthrene in sesame oil into 60 day old mice of the NH descent have been published (25-28, 30). It is now intended to examine more in detail the various types of tumors obtained in mice of the early generations of inbreeding following the original outcross between the CBA, N, and JK strains by which the NH and NHO sublines were established.

MATERIALS AND METHODS

The NH strain was originally established as a selective group of mice following tandem crosses between animals of the CBA, N, and JK strains (25). During the early generations (F_4-F_7) following establishment of the NH stock, groups of mice at 60 days of age received subcutaneous injections of 1 mgm. of methylcholanthrene (Eastman) dissolved in 0.1 cc. of sesame oil (25). Mice of the NH stock that were treated in this fashion have been termed NHO's. Therefore, all the mice included in this report were animals from the NH strain of the F_4-F_7 generations that had been injected with methylcholanthrene as described above. Every effort was made to prevent contamination of the skin by the carcinogen. The needle was inserted in the subcutaneous tissues for its entire length and the remaining carcinogen and oil was cleaned from its surface after each injection. Upon withdrawal of the needle the puncture point was pinched together with the fingers for a short time to prevent leakage. The tissues were fixed in Bouin's solution. Sections were cut perpendicular to the body surface near the greatest diameter of the tumor. At least 4 sections were made through various parts of each tumor. Tissues were stained routinely with hematoxylin and eosin. In some instances additional sections were stained by Masson's trichrome method and with phosphotungstic acid hematoxylin.

Bronchiogenic carcinoma is the only spontaneous new growth to which the NH stock shows any significant susceptibility. These pulmonary tumors are limited to animals older than 14 months. A very small number of mammary neoplasms and leukemias have been observed. However, the incidence of all types of spontaneous tumors with the exception of those of the lung is less than 1 per cent. The present study has shown sarcomas of the subcutaneous connective tissue and epidermoid carcinomas to be the growths most frequently induced in NH mice by a single subcutaneous injection of methylcholanthrene in sesame oil. These two types of neoplasm have never been observed to occur spontaneously in mice of this stock.

Types of Tumors and Occurrence

Included in this report are 694 animals that were killed for study when palpable tumors (approximately 1 cm. in size) were observed on the body surface. Eighty per cent of the NHO mice that received a single subcutaneous injection of methylcholanthrene developed tumors, and no significant sex difference existed as to their occurrence (27). Table I presents the types of neoplasm observed and information relevant to their occurrence. As is usual following the subcutaneous injection of methylcholanthrene, the predominant type of tumor was the fibrosarcoma, but the epidermoid carcinoma is the growth to which major attention has been devoted in this study. All these were squamous cell carcinomas of the skin.

The epidermoid carcinomas were second in the order of frequency, appearing in slightly over 34 per cent of the tumor-bearing animals (Table I). The latent period (subsequent to the injection of methylcholanthrene) of the skin tumors was relatively short, and if the total number of animals that developed

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epidermoid carcinomas is considered 78.9 per cent showed tumors within 150 days after injection (Fig. 1). In the animals that had skin tumors alone, 84.7 per cent were evident upon gross examination prior to 150 days after treatment. Skin tumors alone were observed as early as 58 days after treatment and none later than 230 days, although some of the animals survived for 850 days after having been injected with methylcholanthrene. The average latent period for the induction of epidermoid carcinomas, based upon animals with no other tumor, was 115 days. Combined squamous cell carcinomas of the skin and fibrosarcomas were present as early as 64 days and as late as 496 days after injection. In such animals it was, of course, impossible to determine which tumor appeared first. Fibrosarcomas alone appeared as early as 56 days and as late as 338 days. When all the fibrosarcomas are included except those that occurred with skin tumors or mammary tumors, 50.5 per cent appeared within 150 days post injection and the average latent period was 180 days. The average latent period of the combined epidermoid carcinomas and fibrosarcomas was 138 days. These findings show that the epidermoid carcinomas tended to appear earlier than the sarcomas. The other types of tumors occurred too infrequently to allow any discussion of latent period and distribution. The mammary tumors were all adenocarcinomas and were limited to females. Some findings relevant to mammary tumors in NHO mice treated with methylcholanthrene have been previously reported (30).

Histopathology of the Tumors

As stated above, all the epidermoid tumors were squamous cell carcinomas (acanthomas). All showed considerable keratinization, and more than one-half were associated with inflammatory changes ranging from diffuse collections of polymorphonuclear leukocytes to abcess formation and ulceration accompanied by excessive cornification (Fig. 2); however, inflammatory lesions were not a necessary concomitant. In a few instances the tumors formed papillomatous projections at the surface, but more often extended inward and invaded the derma, subcutaneous fascial layers, and muscle. Many of these epithelial tumors had a "stroma" consisting of a fibrosarcoma. At least one-half of the focal areas of tumor formation were associated with inflammation, cornification, and ulceration. All the cells of the skin tumors showed some degree of keratinization. This was true for the dermal, fascial, and intramuscular extensions of the tumors as well as for the papillomatous projections or masses. In these tumors pearl formations or cell nests were rather numerous. The tumors were malignant in that they invaded all underlying connective tissue and muscular elements, and in many cases the fibrosarcomas. Mitoses of basal layer-like cells were numerous. In some in-

Table I

Number of animals	Sex	Classification of tumors	Average latent period, days	Minimum and maximum period for tumor induction
37	Male	Epidermoid carcinoma	112	58-230
48	Female	Epidermoid carcinoma	118	59-226
70	Male	Epidermoid carcinoma and fibrosarcoma	136	65-496
74	Female	Epidermoid carcinoma and fibrosarcoma	139	64-488
3	Female	Epidermoid carcinoma and mammary carcinoma	119	107-134
6	Female	Epidermoid carcinoma, mammary carcinoma, and fibrosarcoma.	160	84-229
1	Male	Epidermoid carcinoma and bronchiogenic carcinoma	—	210
3	Female	Epidermoid carcinoma and bronchiogenic carcinoma	283	134-452
227	Male	Fibrosarcoma	151	80-309
130	Female	Fibrosarcoma	142	56-276
19	Female	Fibrosarcoma and mammary carcinoma	157	85-253
3	Male	Fibrosarcoma and bronchiogenic carcinoma	288	272-309
1	Female	Fibrosarcoma and bronchiogenic carcinoma	—	283
45	Male	Hypertrophied epidermis, showing in some cases inflammation and keratinization, plus a fibrosarcoma	147	70-300
24	Female	Same as above	148	96-338
3	Male	Epidermis same as above, but with a fibrosarcoma and bronchiogenic carcinoma	124	99-144

Total number of animals with tumors	694
Total number of animals with epidermoid carcinoma	242
Total number of tumors	883
Percentage of tumor-bearing animals with epidermoid carcinoma	34.7

stances the regional nodes showed metastases. When an animal also had a mammary tumor that had undergone squamous metaplasia it was difficult to determine whether the source of the metastasis was mammary tissue or skin. The lymph node shown in Fig. 8 is from an animal that had no mammary tumor.

In addition to the squamous cell carcinomas, 72 of the animals that showed fibrosarcomas had a definitely thickened skin covering these tumors. This hyperplasia of the epidermis was in some instances associated with inflammation and hyperkeratinization, in others not. In the latter the epidermis was similar to that shown in Fig. 4. Of the 72 animals with thickened skins, 44 (61 per cent) were killed at less than 150 days subsequent to treatment. Such thickened skins were not observed except over sarcomas since animals were not killed until they developed a palpable tumor. As a result of this, true early lesions were not obtained from animals in which only skin tumors developed. Therefore the opportunity to observe stages in the growth and extension of the epidermoid carcinomas was obviously limited to adjacent areas of the skin of animals that had developed relatively large tumors of the epidermis. In such areas all the epidermal elements seemed to contribute to the neoplastic processes. Not only was there hyperplasia of the germinal layers of the skin, but hyperplasia and squamous metaplasia were observed in the hair follicles and sebaceous glands (Figs. 5 to 7). Also, many of the mammary tumors showed squamous metaplasia associated with inflammation and excessive keratinization (30) Some of the further extensions of the neoplasms consisted of an even and uniform downgrowth of the basal layer of the epidermis (Figs. 3 and 4). Others were formed by projections along hair follicles (Figs. 6 and 7). It is incorrect to describe the hair follicles as passive routes in the process of neoplasia since the cells of these structures appeared to undergo hyperplasia and keratinization. Although the tumors consisted almost entirely of large squamous cells, the

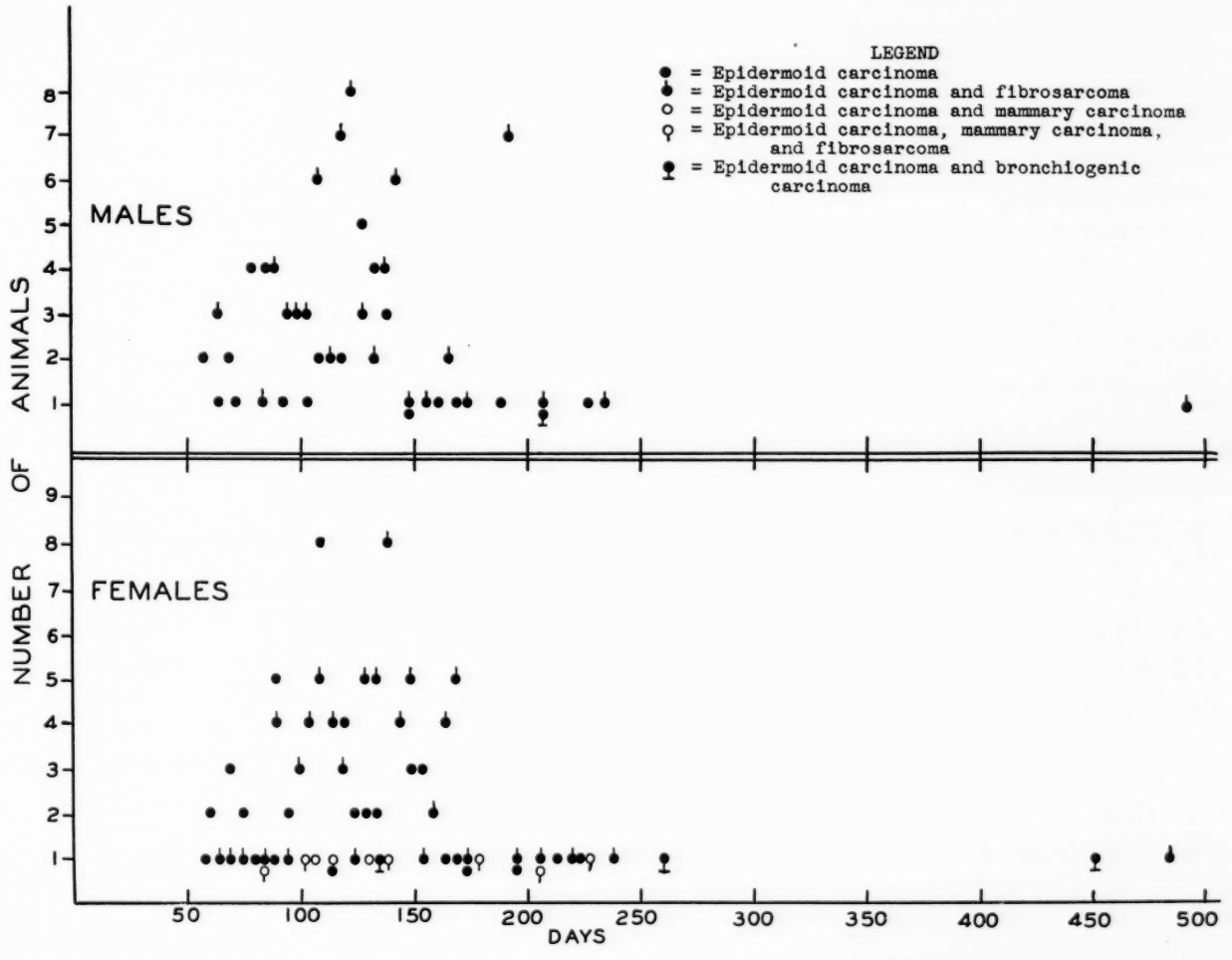
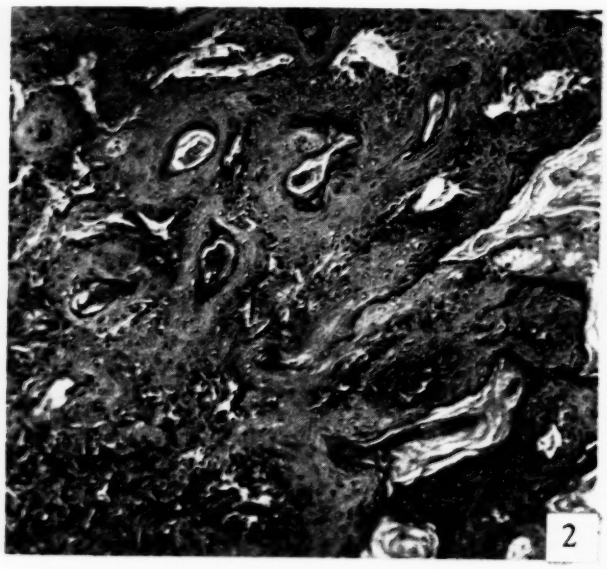
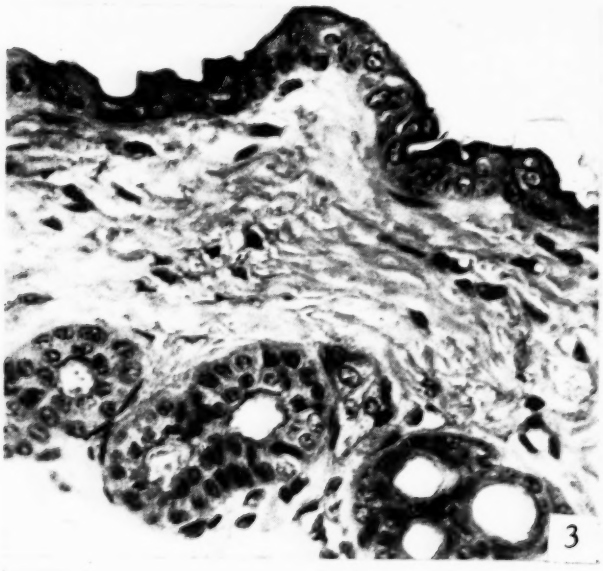
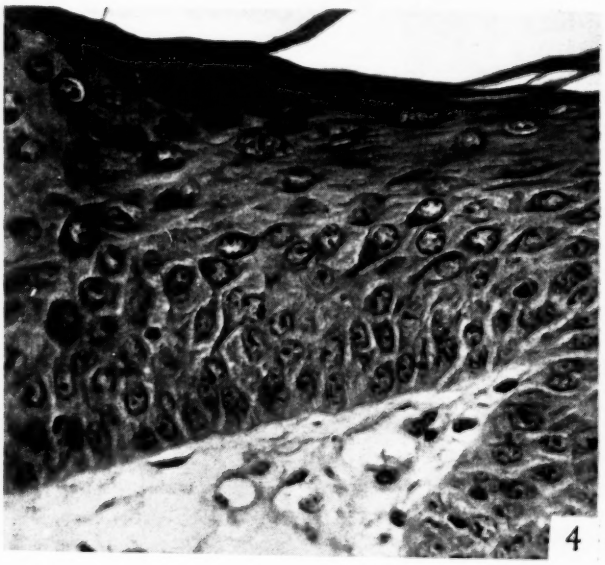
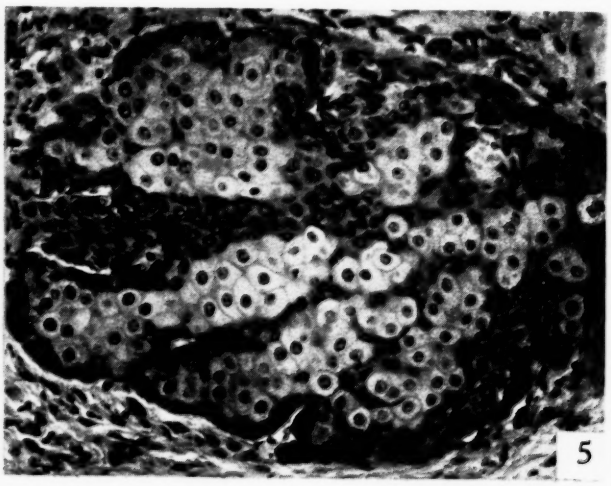


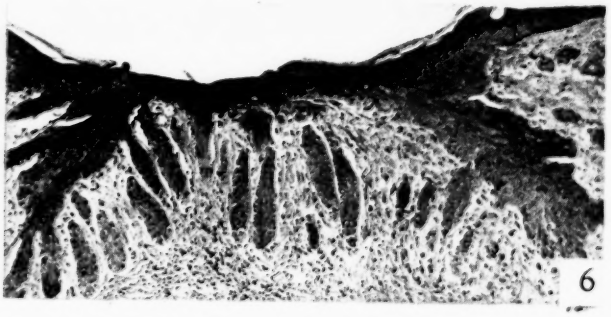
FIG. 1.—Number and distribution of epidermoid carcinomas and associated tumors in NH mice that received a single subcutaneous injection of 1 mgm. of methylcholanthrene in 0.1 cc. of sesame oil at 60 days of age. "Days" represents the period subsequent to injection at which the tumors were observed to be present.

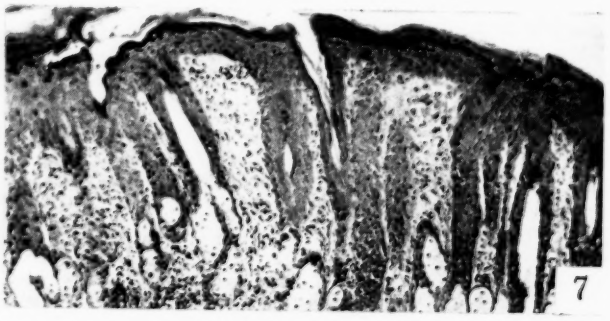


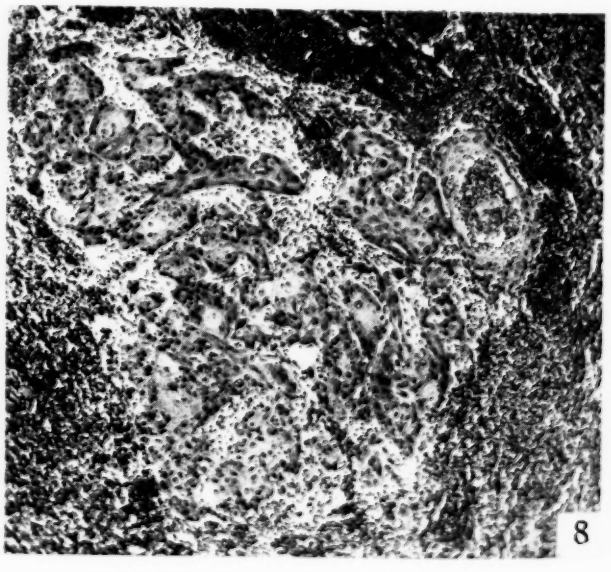












FIGS. 2-8

ones that seemed to result from uniform downgrowths of basal epidermal cells were less keratinized than the others.

The sarcomas consisted mostly of spindle-shaped fibrocytes and collagenous fibers. Mitoses of fibroblast-like cells were very numerous. A very small number of the sarcomas can be described as undifferentiated, with relatively little evidence of fiber formation. In general the sarcomas were the typical fibrous tumors common to the subcutaneous connective tissue of animals that have been injected with carcinogenic hydrocarbons.

DISCUSSION

For the most part the experimental production of skin tumors has been based upon the technic of applying solutions of a carcinogen in benzene or acetone directly to the epidermis. Protracted painting has been by far the most frequent method (4, 8, 12, 24, 29). Fieser (12) has stated "in order to produce skin tumors in mice with even a highly potent carcinogen in benzene solution some thirty deliberate applications must be made." More recent work, however, has shown this not to be true in certain strains of mice (10, 15, 17, 18). In the earlier experiments skin tumors were produced by applying coal tar and its distillates directly to the surface (21, 31). The morphological changes associated with the development of skin tumors by the methods just mentioned have been described in considerable detail (7, 9, 19).

It is well known that sarcomas constitute the predominant type of tumor following the subcutaneous injection of several of the carcinogenic hydrocarbons. New growths of the epidermis and mammary glands are relatively infrequent in occurrence (1, 11, 22). In the present series of 694 animals (all with tumors) 34.7 per cent developed squamous cell carcinomas of the skin. Shimkin and Andervont (22) reported that there were only 4 squamous cell carcinomas among the 260 tumors that developed in C3H mice subsequent to the subcutaneous injection of methylcholanthrene, dibenzanthracene, or benzpyrene (all dissolved in tricaprylin). Burdette and Strong (6), using 5 inbred strains of mice, found that a single subcutaneous injection of methylcholanthrene induced only a small number of epidermoid carcinomas and that those were limited to mice of the CBA strain. Mider and Morton (17) have reported the production of a considerable number of papillomas and a much smaller number of epidermoid carcinomas by single painting of the skin of C57 brown mice with methylcholanthrene in benzene. Later (18) these same workers observed that a single painting produced only benign papillomata in C57 black mice, and that multiple paintings caused some of the papillomas of both C57 browns and C57 blacks to become malignant. Similar findings have been reported for C57 brown mice receiving single or multiple paintings with 9,10-dimethyl-1,2-benzanthracene (15) and for Swiss mice painted in a like fashion with methylcholanthrene (10). By brother-to-sister mating of the descendants of mice that were relatively susceptible to "tar warts" strains have been developed that showed a high incidence of epidermoid tumors when tar or its distillates was applied directly to the surface (2, 14, 19). It has been observed that two strains of mice, one with a high susceptibility and the other with a low susceptibility to spontaneous mammary tumors, responded uniformly to the production of skin tumors by means of protracted tarring (20). However, several reports have indicated that certain strains of mice possess a relatively high susceptibility to the induction of skin tumors by the direct epidermal application of carcinogens for a protracted period (5, 13, 16). It has not been demonstrated that the same susceptibility exists in these strains when the carcinogen is injected subcutaneously. On the contrary, mice of the IF stock, which are highly susceptible to carcinogenic agents applied directly to the skin, developed fewer epidermal tumors subsequent to the subcutaneous injection of methylcholanthrene than did several other strains (3).

In rats epidermoid tumors seem to occur only rarely as the result of subcutaneously injected methylcholanthrene (11). Guinea pigs receiving a single injection of methylcholanthrene or 4 injections at 2 week intervals apparently did not develop any epidermoid carcinomas (23).

The high frequency of occurrence prior to 150 days after treatment (84.7 per cent of the tumor-bearing animals with epidermoid carcinomas alone) and the relatively short average latent period of 115 days showed that the skin tumors developed earlier than

DESCRIPTION OF FIGURES 2 TO 8

FIG. 2.—Typical squamous cell carcinoma of skin. Mag. × 85.

FIGS. 3 and 4.—Normal and thickened areas of skin from the same mouse. Mag. × 425.

FIG. 5.—Sebaceous gland showing metaplastic changes. The periglandular connective tissue showed no evidence that the gland was being invaded by tumor cells. Mag. × 300.

FIGS. 6 and 7.—Areas adjacent to squamous cell carcinomas. Note hyperplasia of squamous cells along hair follicles. Mag. × 85.

FIG. 8.—Metastasis from an epidermoid carcinoma in a subcutaneous lymph node. The capsule of the node had not been directly invaded. Mag. × 75.

the sarcomas (50.5 per cent prior to 150 days and an average latent period of 180 days). The average latent periods and the chronological distribution of the epidermoid carcinomas and fibrosarcomas, alone and in combination, were essentially the same in both sexes. The mammary tumors were limited to females. Associated with the squamous cell carcinomas of the epidermis were metaplastic processes in the sebaceous glands, hair follicles, and mammary glands that resulted in the formation of large squamous cells and widespread keratinization. Considerable inflammation, abcess formation, and ulceration were frequent but not consistent concomitants of the skin tumors. In some instances the early evidence of epidermal neoplasia was focal, in others relatively generalized.

On the basis of considerable available data it is indicated that the NH mice used in the present study possessed a definite susceptibility for the induction of skin tumors by a single subcutaneous injection of methylcholanthrene. There is no evidence that they are susceptible to the spontaneous occurrence of skin tumors. In relation to the induction of sarcomas and mammary tumors the NH mice showed no fundamental differences from other strains of mice and certain other species.

SUMMARY AND CONCLUSIONS

Tumors developed in approximately 80 per cent of the NH mice that had received a single subcutaneous injection at 60 days of age of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. In the 694 tumor-bearing animals presented here the order of frequency of tumor types was: fibrosarcoma, epidermoid carcinoma, mammary carcinoma, and bronchiogenic carcinoma. The epidermoid growths were all squamous cell carcinomas. These were present in 34.7 per cent of the tumor-bearing animals and tended to occur earlier than the other types. In the animals that showed only skin tumors the average latent period was 115 days and 84.7 per cent of the neoplasms were evident upon gross examination prior to 150 days after injection with carcinogen. For the total group of fibrosarcomas (animals without skin or mammary tumors) the average latent period was 180 days and 50.5 per cent appeared within 150 days. The occurrence of epidermoid carcinomas was greater in the NHO mice than has been reported for other strains of mice and for other rodents treated in a similar manner with methylcholanthrene.

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Progesterone Treatment of Uterine and Other Abdominal Fibroids Induced in the Guinea Pig by Alpha-Estradiol*†

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INTRODUCTION

Uterine fibroids (Nelson, 11) and other abdominal fibroids (Iglesias, 1; Lipschütz and Iglesias, 6) produced in the female guinea pig by prolonged administration of estrogens, can be prevented by the simultaneous administration of other steroids (Lipschütz and Vargas, 9; Lipschütz, 4). Progesterone has been shown to be the most powerful antifibromatogenic steroid in the guinea pig (Lipschütz, Vera, and González, 10).

The question then arises whether it would be possible also to cause regression of already existing experimental fibroids by the subsequent administration of an antifibromatogenic steroid. This seems very probable for the following reasons: first, experimental abdominal fibroids regress when the administration of estrogens is suspended (Lipschütz, Iglesias, and Vargas, 7); and second, the antifibromatogenic action of progesterone and other steroids is concomitant with an antiestrogenic action (9); *i.e.*, it is equivalent to the withdrawal of the estrogen. The antifibromatogenic hormone acts as an antagonist to the estrogen, probably *by desensitizing the reacting tissue*, as suggested in our earlier work on the antagonism of gonadal hormones on the basis of experiments with ovarian grafts in partially castrated males (Lipschütz, 2, 3).

EXPERIMENTS [1]

Pellets, or fragments of tablets, of alpha-estradiol (6 to 18 mgm.) were implanted subcutaneously in 30 castrated female guinea pigs of 310 to 525 gm. About 80 days afterwards, a period sufficient to allow for the growth of abdominal fibroids, tablets of synthetic progesterone (37 to 55 mgm.) were implanted subcutaneonusly. The animals were sacrificed 34 to 52 days after the implantation of progesterone, or 110 to 130 days after implantation of the estrogenic pellet. Pellets of alpha-estradiol (5 to 16 mgm.) were also implanted in 43 castrated females in which no subsequent implantation of progesterone was made. These animals were sacrificed at 80, 110, and 130 days after implantation of the estrogen. The fibrous abdominal reaction or the "total tumorous effect" (T.T.E.) was calculated according to a system explained in former papers. The tumors of four regions—uterus (subserous, mesometrial); mesosalpinx ("apical" tumors); digestive tract and abdominal wall; and spleen (Lipschütz, Iglesias, and Vargas, 8)—are arranged for each region separately in four classes according to their size (Lipschütz, Bellolio, Chaume, and Vargas, 5). The classes are characterized by the values 0.5, 1 (1.5 to 2.5 mm.), 2 (3 to 5.5 mm.), and 3 (6 mm. or more). The T.T.E. is the sum of these four regional values. Fibrous peritoneal strands in the four different regions are characterized by the value 0.5, and exceptionally by 1. Though the size of tumors is not measured, but roughly estimated, and though their shape is not always spherical, the classification gives fairly reliable data, as has been demonstrated by numerous comparative tests in this department in the course of the last four years.

The results, summarized in Table I, show that the tumorous effect was less pronounced in those animals into which a tablet of progesterone had been subsequently implanted and allowed to act for a certain time. The difference between the groups with estradiol alone (A,B,C) and those with estradiol and progesterone (D,E) is considerable (Figs. 1 to 4). This difference might be due to the interaction of two phenomena: first, *prevention* of further neoplastic growth, and second, *regression* of already existing neoplastic growth. The participation of each can be calculated by comparing the different groups (Fig. 5). The aver-

* Because of the difficulties of international communication the authors have not read proof of this article.

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[1] The experimental data were taken from a thesis presented by Dr. Maas in 1942 for the degree of M. D. in the Universidad de Chile, under the title "Ensayo de Tratamiento del Fibromioma Experimental con Progesterona." Various corrections were made in the classification of tumors. The senior author is responsible for the interpretation of results in the present paper.

age T.T.E. in A (the 80 day estradiol group) was 4.0; and in D and E (the groups that after an estrogenic action of 80 days underwent the action of progesterone also) was only 2.5 and 2.2. This is a regression of 37 progesterone was added. Uterine bleeding was frequent in the estradiol period; none occurred in the progesterone period in those animals in which the vagina remained open or opened transiently.

TABLE I

Group	Number of animals (1)	Duration of experiment: Estradiol alone, days (2)	Duration of experiment: Estradiol and progesterone, days (3)	Quantities absorbed per day: Estradiol, μgm. (4)	Quantities absorbed per day: Progesterone, μgm. (5)	Total tumorous effect (T.T.E.), average (6)	Significant difference of average T.T.E. (7)	Number of animals with T.T.E. not less than 4: Total (8)	Number of animals with T.T.E. not less than 4: Per cent (9)	T.T.E. of all animals of the group (for details see Table II, columns 3 and 7) (10)
A Estradiol	27	74–86	0	36 (9–69) *	0	**4.0±0.53** †	—	13	**48**	0.5; 1; 1; 1.5; 1.5; 1.5; 1.5; 2; 2; 2.5; 2.5; 3; 3.5; 3.5; 4; 4.5; 5; 5.5; 5.5; 6; 6.5; 7; 7; 7.5; 8; 8; 11
B Estradiol	5	110–114	0	33 (22–46)	0	6.2	3.5 ‡	5	94	5.5; 6; 6; 6.5; 7
C Estradiol	11	130	0	45 (32–58)	0	7.2	**7.0±0.67**	10		3; 3.5; 4; 5; 5.5; 8; 8; 9; 9; 12; 12
D Estradiol and progesterone	14	74–79	34–38	32 (22–62)	280 (229–332)	2.5	2.9 §	3	**17**	0.5; 1; 1; 1; 1.5; 1.5; 1.5; 2.5; 2.5; 3; 3.5; 4; 4.5; 6.5
E Estradiol and progesterone	16	79–81	50–52	43 (18–73)	257 (204–295)	2.2	**2.3±0.26**	2		1; 1; 1; 1; 1.5; 1.5; 1.5; 1.5; 2; 2; 2.5; 3; 3.5; 3.5; 4; 4.5
						A=100 D+E= 58		=100 = 36		

* Figures in parentheses indicate range.

† $\epsilon = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$ (standard deviation of the average).

‡ $\frac{m_{B+C}-m_A}{\sqrt{\epsilon^2_{B+C}+\epsilon^2_A}}$ (significant difference); m=average.

§ $\frac{m_A-m_{D+E}}{\sqrt{\epsilon^2_A+\epsilon^2_{D+E}}}$

TABLE II

Group	Total number of animals (1)	Animals with some abdominal fibrous reaction, per cent (2)	With regional tumorous marks 1, 2, and 3: Number of animals (3)	With regional tumorous marks 1, 2, and 3: Percentage of animals (4)	Number of regional tumorous marks 1, 2, and 3: Total (5)	Number of regional tumorous marks 1, 2, and 3: Per animal of column 1 (6)	With regional tumorous marks 2 and 3: Number of animals (7)	With regional tumorous marks 2 and 3: Percentage of animals (8)	Number of regional marks 2 and 3: Total (9)	Number of regional marks 2 and 3: Per animal of column 1 (10)
A	27	**100**	21	**78**	50	**1.85**	14	**52**	33	**1.22**
B	5	100	5	100	16	3.25	5	94	13	2.42
C	11	100	11		36		10		26	
D	14	**100**	7	**53**	14	**0.97**	4	**20**	5	**0.23**
E	16	**100**	9		15		2		2	
	A	100	—	100	—	100	—	100	—	100
	D+E	100	—	68	—	52	—	38	—	19

and 45 per cent respectively. As can be seen from Fig. 5, the amount of regression of the T.T.E. was almost equivalent to that of prevention, as calculated by comparing A with B and C. Regression was thus as significant statistically as was prevention (see Table I, column 7).

Other antiestrogenic actions also were evident. The vagina, which was open throughout the estradiol period, closed again in the majority of animals when

Behavior of Abdominal Fibroids

Since the T.T.E. is the sum of four values, and since fibrous strands valued 0.5 also are included in this sum, it is evident that a T.T.E. of 2.0 can be reached in some cases in which only minute tumors or fibrous strands valued 0.5 are present without a single tumor of 1.5 mm. or more (classes 1 to 3) being found. As seen from Table I, column 10, and Table II,

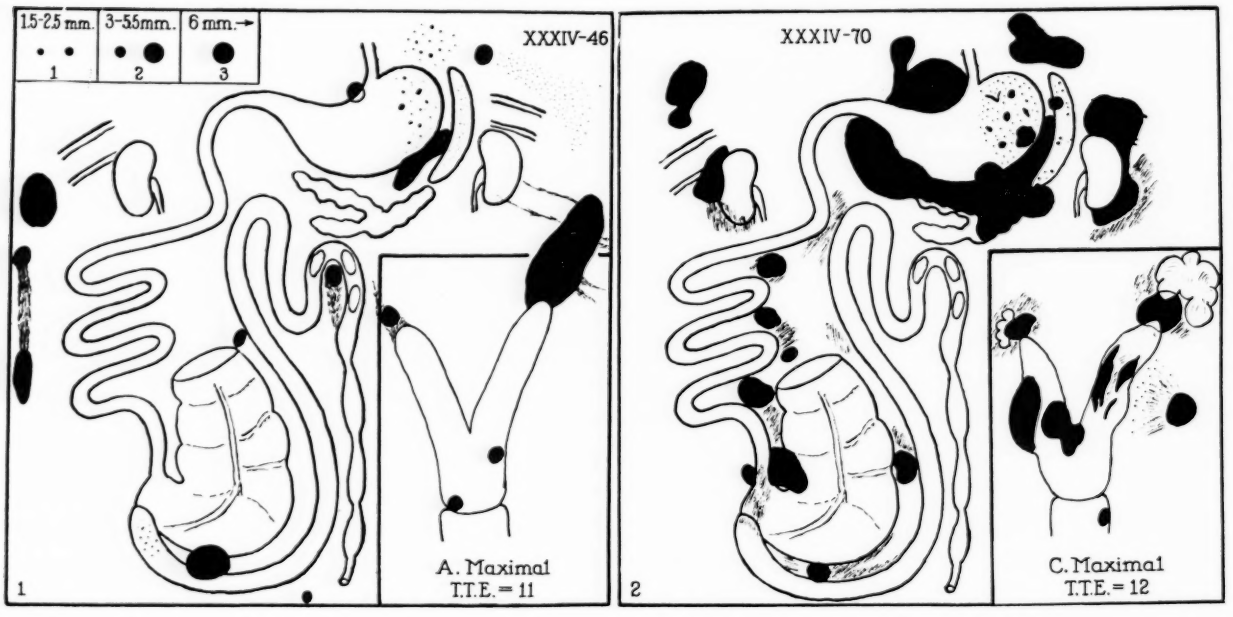

FIG. 1.—Animal of group A (XXXIV, 46); 84 days; 69 μgm. of estradiol daily. Maximal reaction, T.T.E. = 11. Uterine tumors, 2; apical tumors, 3; tumors of digestive tract and abdominal wall, 3; tumors in the hilum of the spleen, 3.

FIG. 2.—Animal of group C (XXXIV, 70); 130 days; 34 μgm. of estradiol daily. Maximal reaction, T.T.E. = 12.

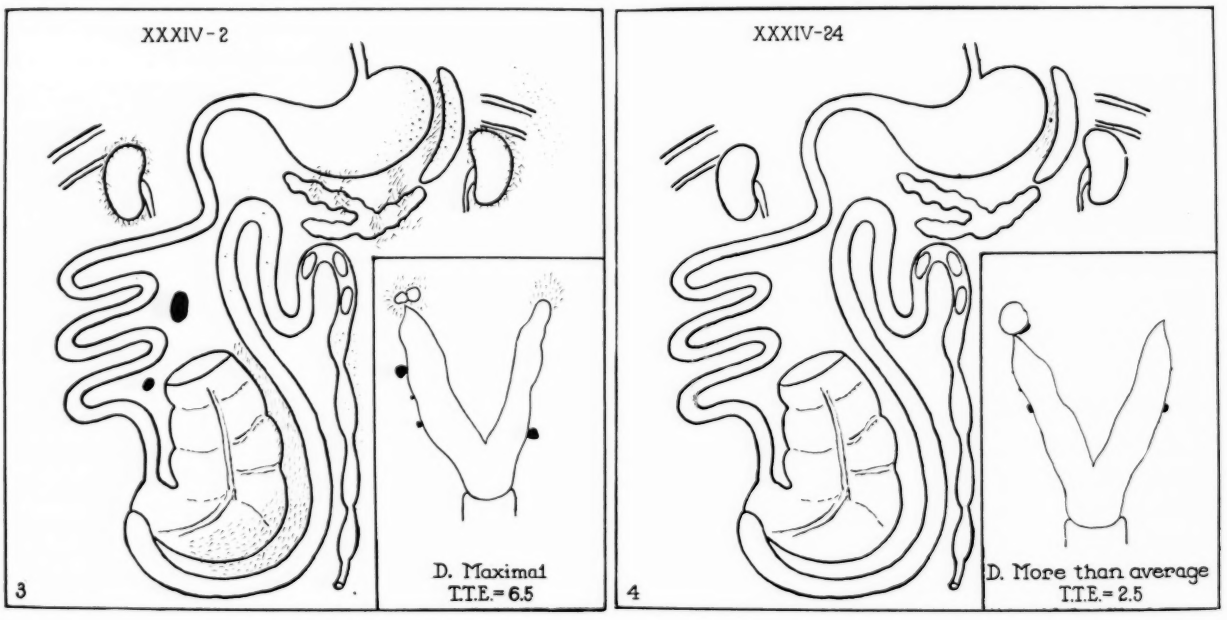

FIG. 3.—Animal of group D (XXXIV, 2); 115 days with estradiol; 37 μgm. of estradiol daily; 37 days with progesterone; 292 μgm. of progesterone daily. Maximal reaction, T.T.E. = 6.5. Uterine tumors, 2; apical fibrous strands, 0.5; tumors of the digestive tract, small nodules ("tumorous seeds") on the stomach and abdominal wall, fibrous strands, 3; multiple nodules on the spleen, 1.

FIG. 4.—Animal of group D (XXXIV, 24); 111 days with estradiol; 32 μgm. of estradiol per day; 35 days with progesterone; 309 μgm. of progesterone per day. More than average reaction, T.T.E. = 2.5. Uterine tumors, 1; apical tumors, in the wall of cystic tube, 1; tumor in the hilum of the spleen, 0.5.

column 2, *all* animals of groups D and E showed some abdominal fibrous reaction which had not been suppressed by the subsequent action of progesterone. It may be remembered that in our former work with the *simultaneous* implantation of fibromatogenic and antifibromatogenic pellets a certain amount of abdominal fibrous reaction was present also, although induction of tumors of classes 1 to 3 was prevented in most animals (9). This makes it very probable that regression under the influence of an antifibromatogenic steroid might be made more evident if stress were laid on tumors not smaller than class 1 (1.5 to 2.5 mm.) and especially on those belonging to classes 2 and 3

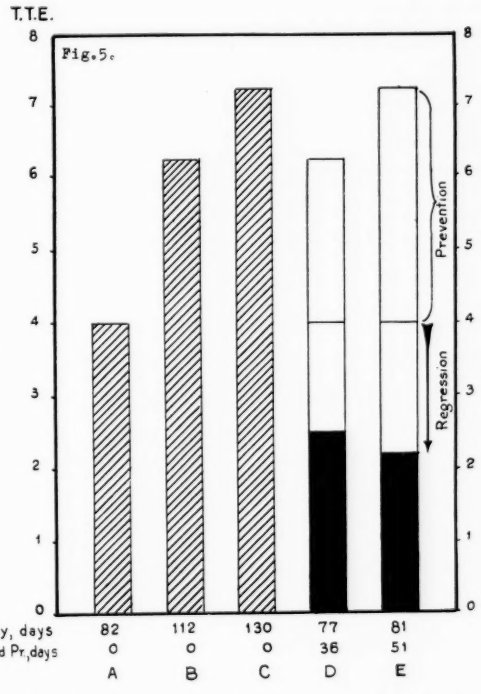

FIG. 5.—Total tumorous effect (T.T.E.) of the 5 groups of Table I. The T.T.E. is smaller in D and E than in A. This shows that there has been *regression* of existing abdominal fibroids under the influence of progesterone.

(size of a pea or greater; see Fig. 1). This has been done comparatively under the headings (a), (b), and (c).

(a) The average T.T.E. in D and E taken together indicates a regression of 42 per cent, as compared with A (Table I, column 6). However, the question is relevant *in how many animals* the average T.T.E. of 4.0 of group A has been maintained in the different groups; this average T.T.E. of 4 is due to the presence of tumors of classes 1 to 3 accompanying the less significant signs of fibrous reaction. The T.T.E. of 4.0 was found in only 5 of 30 animals of groups D and E (Table I, columns 8 and 10), whereas 13 out of 27 animals of group A reached this value. This means 17 per cent in D and E instead of 48 per cent in A, or that the regression of the relative frequency (the figure for A taken as 100) of animals with larger fibroids amounted to 64 per cent (Table I, column 9). This quantitative result is shown also in a more direct way by the following data.

(b) There was no *lowering* of the frequency of animals with some fibrous abdominal reaction (Table I, column 10; Table II, column 2). However, a difference becomes obvious when the frequency of animals with tumors not smaller than class 1 is considered. There were 78 per cent of animals with tumors of classes 1, 2, and 3 in group A (Table II, column 4) and only 53 per cent of such animals in groups D and E. The lowering of the relative frequency (A = 100) of animals with these tumors amounts to 32 per cent. More striking evidence is presented by considering only animals with tumors of classes 2 and 3. At 80 days, the frequency of animals with tumors of classes 2 and 3 was 52 per cent in group A (Table II, column 8). With a subsequently implanted pellet of progesterone in groups D and E, the frequency dropped to 20 per cent in the course of 5 to 7 weeks. This means that the lowering of the relative frequency (A = 100) of animals with tumors of classes 2 and 3 amounted to 62 per cent.

(c) An animal may have simultaneously *several* regional marks of classes 1, 2, and 3, according to different localizations of tumors, as indicated above. If the total number of regional marks 1, 2, and 3 in the different groups is considered, the frequency of marks 1, 2, and 3 per animal (Table II, column 6) drops from 1.8 in group A to 1.0 in groups D and E. The relative frequency per animal was 48 per cent smaller in D and E than in group A. When only tumors of classes 2 and 3 are considered (Table II, column 10) the frequency per animal in group A is 1.2, and in group D and E only 0.23; the regression of the relative frequency of these tumors per animal (A = 100) was 81 per cent.

Results as calculated in (a), (b), and (c) show that regression under the influence of progesterone is expressed primarily in a *diminution of the relative frequency of large tumors per animal.* A graphic presentation of these results is given in Fig. 6.

Behavior of Uterine Fibroids

A question deserving of special interest is whether uterine fibroids may behave differently from other abdominal fibroids as regards regression. This is not very probable, since structurally there is no difference between these fibroids. Former experiments with the preventive action of progesterone have shown that the prevention of uterine tumors is complete, whereas some degree of extragenital fibrous reaction might still be present (9); therefore it was thought worth while

in the present work to calculate separately the amount of regression for uterine tumors. Table III and Fig. 7 present our results with reference to uterine tumors, including all classes (0.5 to 3).

Whereas there was some fibrous abdominal reaction in all animals of group A (estradiol alone), a similar reaction, visible on the surface of the uterus or in the mesometrium, was to be found in this group in only 50 per cent of the animals. This is a common finding in our work: the extragenital fibrous reaction is more frequent than the uterine one. In groups D and E (estradiol and progesterone) only 30 per cent of the animals, instead of 59 per cent as in group A, revealed a fibrous uterine reaction. This lowering of the relative frequency of animals with some fibrous uterine reaction (A = 100) thus was 49 per cent. On the contrary, as already pointed out, there was no lowering of the frequency of animals with some fibrous reaction in the abdominal cavity in general (see Table II, column 2, and Fig. 7).

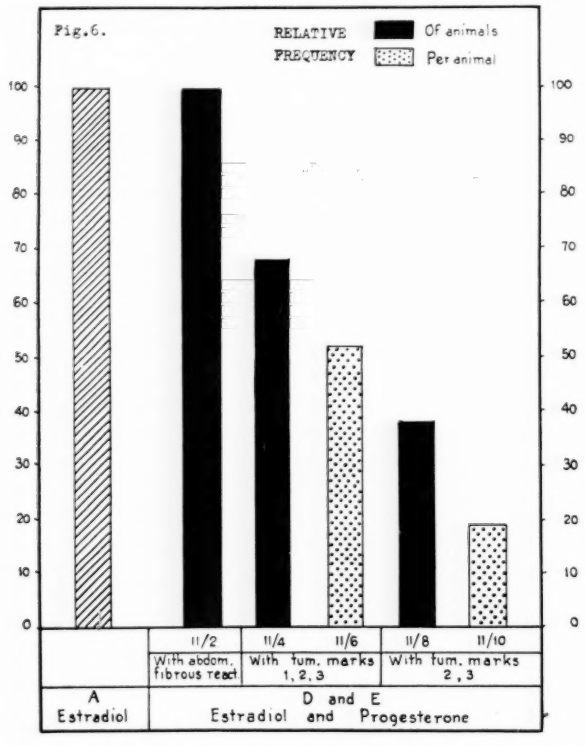

FIG. 6.—Percentage regression of the fibrous effect under the influence of progesterone in groups D and E compared with group A of 100; *i.e.* figures (4), (6), (8), and (10) of Table II for A taken as 100, and figures for D and E correspondingly calculated.

II/2: no regression of frequency of animals with some fibrous abdominal reaction.

II/4: 32 per cent regression of frequency of animals with tumorous marks 1, 2, and 3.

II/6: 48 per cent regression of frequency of tumorous marks 1, 2, and 3 per animal.

II/8: 62 per cent regression of frequency of animals with tumorous marks 2 and 3.

II/10: 81 per cent regression of frequency of tumorous marks 2 and 3 per animal.

When a sufficient number of animals is available, calculation of the frequency of regional tumorous marks 2 and 3 seems to be the most convenient comparative means of appreciating the antifibromatogenic action of steroids.

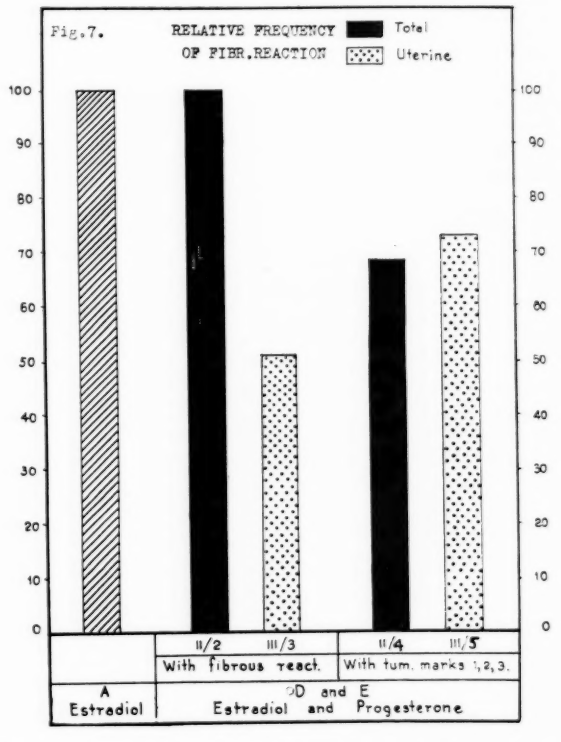

FIG. 7.—Comparative regression of *total abdominal* fibrous reaction (black columns), and of *uterine* fibrous reaction (dotted columns), under the influence of progesterone. All figures compared with group A = 100.

II/2 and III/3: relative frequency of animals with some fibrous reaction (marks 0.5, 1, 2, and 3), abdominal and uterine respectively.

II/4 and III/5: relative frequency of animals with marks 1, 2, and 3, abdominal and uterine respectively.

The frequency of animals with uterine reactions decreases more considerably than the number of animals with abdominal reactions in general (II/2 and III/3). However, there is no significant difference as to the regression of frequency of animals with larger abdominal or uterine tumors (II/4 and III/5).

The frequency of animals with larger uterine tumors of classes 1, 2, and 3 (Table III, columns 4 and 5) was 37 per cent in group A and 27 per cent in groups D and E. This decrease in the relative frequency (A = 100) of animals with uterine tumors, amounting to 27 per cent, was coincident with the decrease in abdominal fibroids in the same classes which was 32 per cent (see Table II, column 4). In other words, the initial fibrous reaction of the uterine peritoneum or of the mesometrium might regress, as seen above, more readily than the general abdominal reaction (Fig. 7, II/2-III/3); but there is no difference

between uterine and abdominal regression when only larger fibroids are considered (Fig. 7, II/4-III/5).

DISCUSSION

The regression of abdominal and uterine fibroids under the influence of progesterone was incomplete in 7 weeks, as explained in Tables I to III and Figs. 3 to 7.

The question then arises whether complete regression might be obtained if progesterone were to act for a longer time. It is not likely that insufficient quantities of progesterone were given in our experiments. As seen from Table I, 200 to 300 μgm. were absorbed per day, yet quantities 5 and possibly even 10 times smaller are sufficient to prevent abdominal fibroids elicited by estrogens, as has been shown recently (10). It is very probable that a more advanced

TABLE III

Group	Total number of animals (1)	With some uterine fibrous reaction (marks 0.5, 1, 2, and 3)		With uterine marks 1, 2, and 3	
		Number of animals (2)	Percentage of animals (3)	Number of animals (4)	Percentage of animals (5)
A	27	16	**59**	10	**37**
B	5	3	75	3	69
C	11	9		8	
D	14	5	**30**	4	**27**
E	16	4		4	
	A	—	100	—	100
	D+E	—	51	—	73

stage of regression, as expressed in the relative frequency of large tumors per animal and in the degree of hyalinization, will be obtained when the antifibromatogenic progesterone is allowed to act for a longer time.

SUMMARY

Abdominal fibroids produced in the female guinea pig in the course of 80 days by subcutaneously implanted pellets of alpha-estradiol *ceased growing* after a tablet of synthetic progesterone was subsequently implanted and allowed to act simultaneously with the estrogen for 34 to 52 days.

Besides this *prevention* of tumorous growth there was, in the progesterone period, also a considerable *regression* of existing fibroids. This is inferred from the fact that the fibrous tumorous effect in animals that were exposed for 80 days to the action of estradiol alone, and subsequently for 34 to 52 days to the action of both estradiol and progesterone, was only about 58 per cent of that found in animals sacrificed after having been exposed during 80 days to the action of estradiol alone.

Regression, *i.e.*, the difference in the average fibromatogenic effect between the estradiol group and the groups treated subsequently with progesterone, was statistically as significant as was prevention.

The therapeutic action of progesterone on existing fibroids elicited by estrogens was revealed to be much more considerable when the *frequency of animals with large abdominal fibroids* and especially the *frequency of large fibroids per animal* were compared in the different groups.

The decrease in frequency of large abdominal fibroids per animal amounted to 81 per cent in 5 to 7½ weeks under the influence of progesterone.

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Microscopic Structure of Estrogen-Induced Uterine and Other Abdominal Fibroids Treated with Progesterone*†

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INTRODUCTION

The fibrous tumorous reaction induced by estradiol in the abdominal cavity of the female guinea pig in the course of two to three months diminishes when the animal is subjected subsequently for one to two months to the simultaneous action of progesterone (Lipschütz and Maas, 7). This regression, which has been demonstrated by a quantitative analysis of our results, can be due only to the *shrinking* of existing fibroids.

So far, the senior author has studied the shrinking of experimental abdominal fibroids only in experiments in which fibromatogenic quantities of estradiol benzoate were administered for about two months and subsequently suspended. The microscopic structure of shrunken fibroids was found to be fundamentally different from that of growing ones. The question arises whether similar structural changes characteristic of shrinking might take place in abdominal fibroids in animals in which diminution of the tumorous reaction was induced not by suspension of the estrogen but by progesterone treatment in the presence of the former. Such microscopic findings would be highly corroborative of regression under the antifibromatogenic influence of progesterone acting in the presence of the fibromatogenic estrogen.

Structure of Fibroids after Suspension of Estrogen

A total of 7 uterine and other abdominal fibroids may serve as examples. These tumors belonged to 4 animals that were treated for several months with injections of estradiol benzoate and sacrificed 1 to 4 months after injections had been suspended.

As described in an earlier paper (8), uterine and other abdominal fibroids are rich in spindle-shaped cells, especially at the periphery (Figs. 13, 17, and 18). These cells, which are probably fibroblasts, are in general separated from one another by collagenous fibers. In the center of the tumor collagenous fibers predominate. Bundles of smooth muscle fibers also may be present, but in rather scant quantities; they are mostly disposed in a "disorderly" manner. When the administration of estrogens is suspended, the fibroids acquire a fundamentally different structure. Similar fibroids, 4 months after the suspension of injections (Figs. 1 and 2, and Figs. 8 and 9), were found

* Because of the difficulties of international communication the authors have not read proof of this article.

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DESCRIPTION OF FIGURES 1 TO 11

Fig. 1.—Mesometric tumor of class 2 (2 × 3 mm.). Castrated female guinea pig (I.8) injected during 8 months, thrice weekly, with 20 μgm. of estradiol benzoate; sacrificed 103 days after the last injection. Van Gieson stain. Mag. × 5.

Fig. 2.—The same tumor. Hyaline condition of the fibrous tissue. Formation of peripheral and central spaces. Mag. × 23. Compare with Fig. 16.

Fig. 3.—The same tumor as in Figs. 1 and 2. Large pseudomedullary spaces in the hyalinized collagenous tissue are cells resembling those of cartilage. Complete absence of spindle-shaped cells. Mag. × 200.

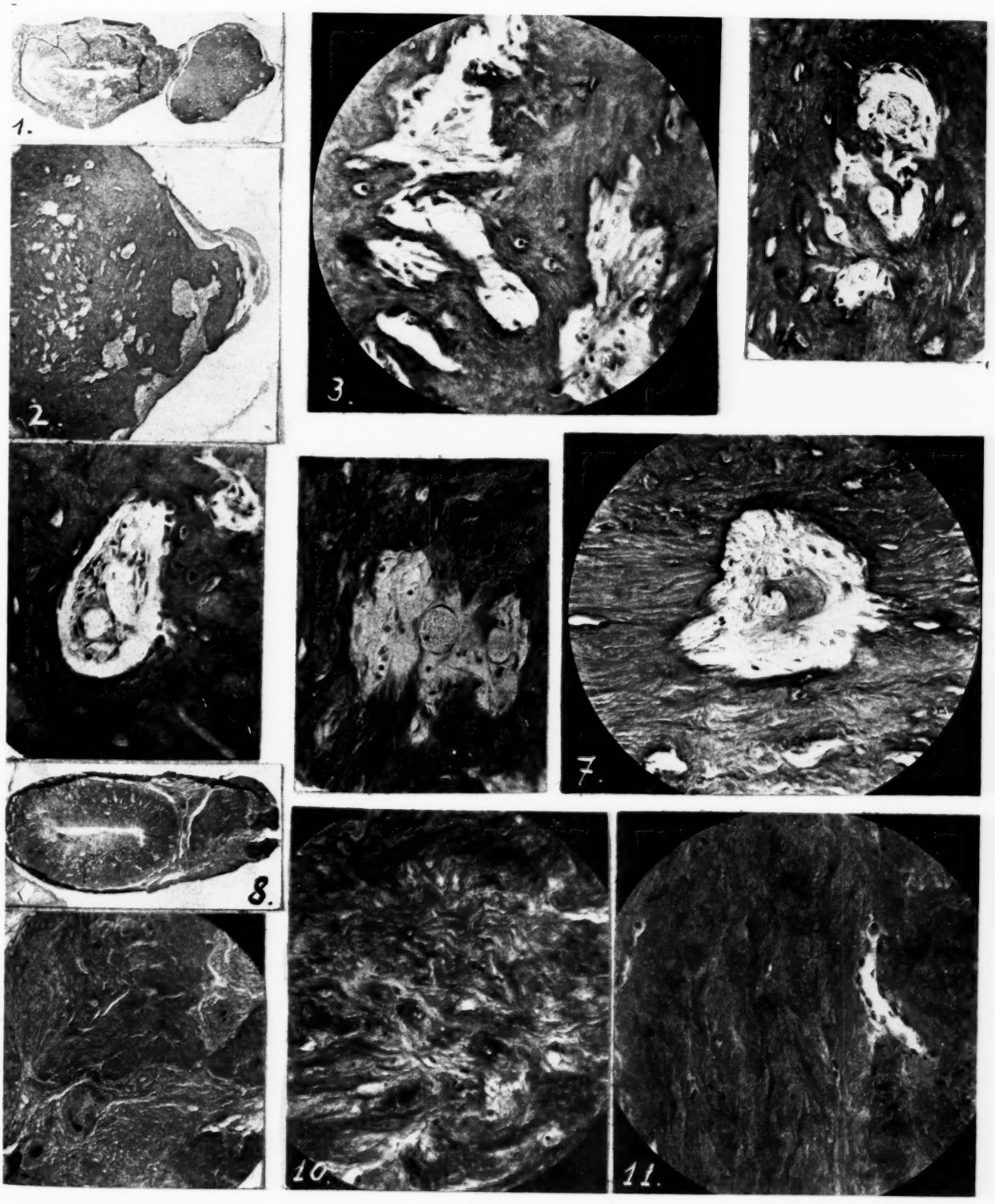
Figs. 4 to 7.—The same tumor. Pseudomedullary spaces with central blood vessels, which in Fig. 7 are in direct contact with the hyaline mass. Cells carpeting pseudomedullary spaces in Fig. 4 and especially Fig. 5. Mag. × 200.

Fig. 8.—Intramural and mesometric tumors of castrated female guinea pig (I.14) injected during 8 months, thrice weekly, with 20 μgm. of estradiol benzoate; sacrificed 113 days after the last injection. Van Gieson stain. Mag. × 5.

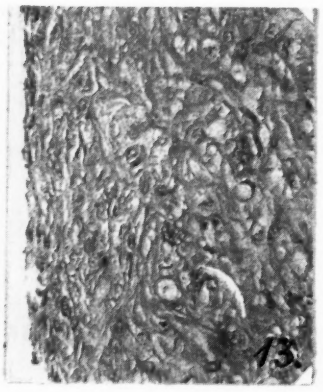
Fig. 9.—The same tumors. The tumor illustrated appears less homogeneous than in Fig. 2. Mag. × 23.

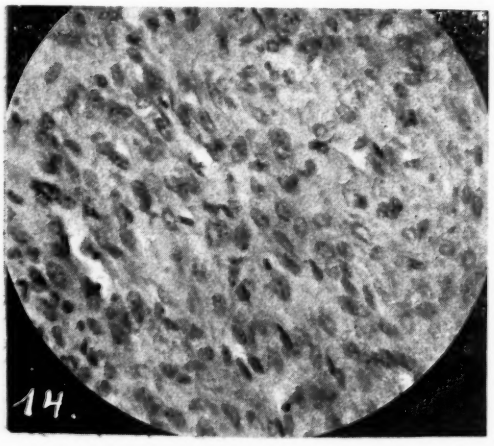
Fig. 10.—Coarse collagenous fibers. Complete absence of spindle-shaped cells. Mag. × 200.

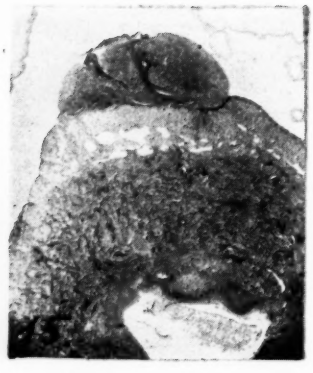
Fig. 11.—Retrorenal tumor of castrated female guinea pig (IX.12) injected during 110 days, thrice weekly, with 80 μgm. of estradiol benzoate; sacrificed 33 days after the last injection. Predominantly collagenous fibers; few scattered small nuclei. Capillary at the right. Complete absence of spindle-shaped cells. Van Gieson stain. Mag. × 200.

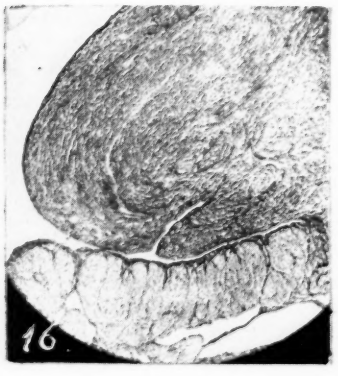


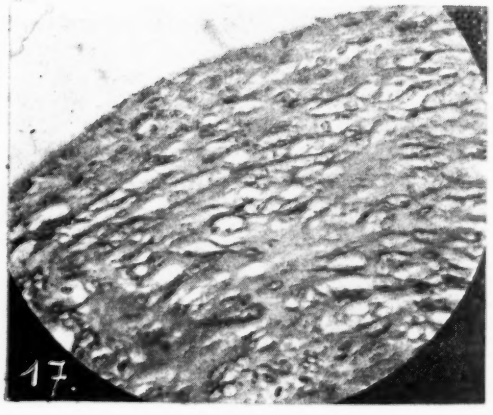
FIGS. 1-11

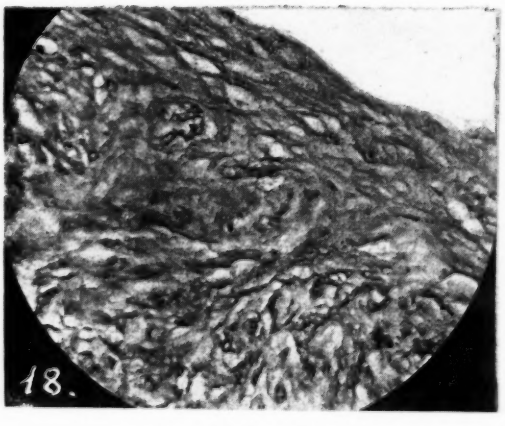












FIGS. 12-19

to be poor in cells (Figs. 7 and 10). The typical spindle-shaped cells of the periphery had disappeared completely. The tumors consisted mainly of hyalinized collagenous tissue. In one case the fibers were very coarse (Figs. 7 and 10). Cells were scarce and scattered between the fibers without any preference for the periphery. The nuclei of these cells were small (Fig. 10), and the protoplasm was scanty. The cells were surrounded by a clear space separating them from the fibrous mass of the tumor (Figs. 3, 4, and 5). The impression was of a fibrous cartilage. This impression was strengthened in one case of a uterine tumor in which local destruction of the hyalinized fibrous tissue took place with subsequent formation of some sort of medullary spaces (Figs. 3 to 7). Local destruction was centered, as in chondriolysis, around small blood vessels, probably capillaries (Fig. 6). The space was sometimes carpeted by a layer of cells comparable to chondroclasts (Figs. 4 and 5). The whole picture coincided as to details with the classic description of the ossification of cartilage as given by Bouin (1) and by Levi (2).

We are unable to say whether or not ossification eventually takes place and how often hyalinization is followed by ossification or calcification, or whether the picture in Figs. 2 to 7 is indicative of a gradual disappearance of the hyalinized tissue. However, there is evidence that the disappearance of fibroblasts and hyalinization begins *early;* collagenous fibers similar to those in Figs. 3 to 7 were found in a retrorenal fibroid of an animal sacrificed 33 days after injections had been suspended (Fig. 11).

The enormous difference between regressing uterine tumors and those that are not regressing, *i. e.,* present in animals given estrogens uninterruptedly until the end of the experiment, is best seen by comparing Figs. 7 and 10 with Figs. 13, 14, and 17 from animals of the C group, described in the previous paper (7), which were treated with estradiol for 130 days. The same is true in comparing other abdominal fibroids (Figs. 11, 18, and 19).

Structure of Fibroids after Progesterone Treatment

Nine tumors were examined. They belong to series D and E of the previous paper (7); *i. e.,* to animals into which tablets of estradiol were implanted subcutaneously on one side of the body, followed 75 to 80 days later by the implantation of tablets of progesterone on the other side. The animals were sacrificed 34 to 52 days after the implantation of progesterone.

Out of 5 *uterine* tumors ranging in diameter between 1 to 3 mm., there were 3 with hyalinization (Figs. 20 and 22). The latter was as typical in these 3 cases as that of tumors shrunk after suppression of estrogens. Hyalinization was less conspicuous in the remaining 2 uterine tumors. However, spindle-shaped cells of the type characteristic of the growing abdominal fibroid were not present in any of these 5 tumors. There were cells with a small nucleus (Fig. 23), as already described in the case of tumors regressing after suppression of estrogens; but their number was larger than in the latter case (Figs. 3 to 7, and Fig. 10).

The comparative description and the figures show clearly that uterine fibroids, induced by an estrogen and found in animals subjected subsequently to the influence of progesterone that is allowed to act in the presence of estradiol, undergo the same changes as do fibroids after the administration of estrogens has been suspended. The extension of hyalinization was apparently less pronounced in the tumors of the present series; but this might be due to the fact that the treatment with progesterone lasted only 34 to 52 days, whereas in our former experiments most animals were sacrificed 3½ months after the administration of estrogens had been suspended.

DESCRIPTION OF FIGURES 12 TO 19

Fig. 12.—Subserous tumor of class 2 (about 2.5 × 5 mm.) of left horn of uterus in castrated female guinea pig (XXXIV.68) with subcutaneously implanted tablet of estradiol. Sacrificed 130 days after implantation; absorption of 39 μgm. of estradiol per day. Van Gieson stain. Mag. × 5.

Fig. 13.—The same tumor. Spindle-shaped peripheral cells. Mag. × 200.

Fig. 14.—The same tumor. Hematoxylin and eosin stain. Large spindle-shaped nuclei. Mag. × 200.

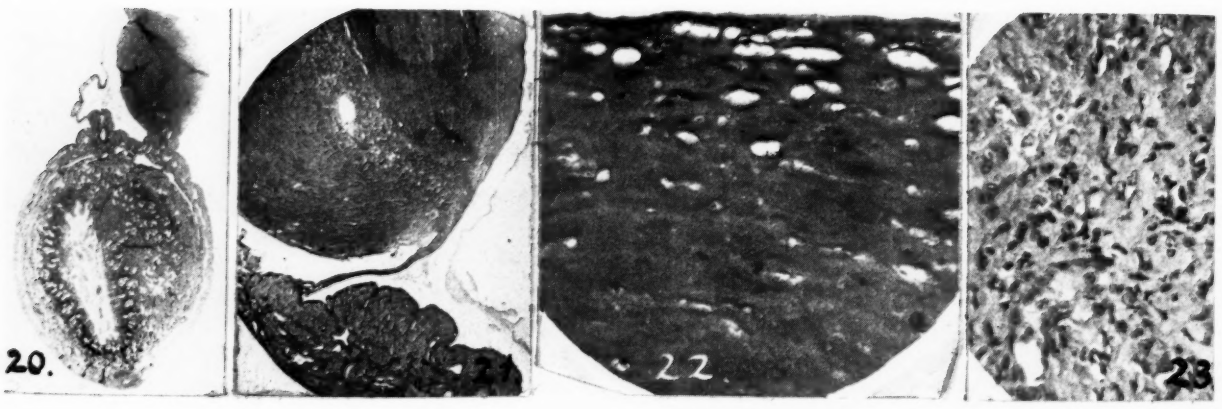
Fig. 15.—Mesometric tumor, class 2 (2 × 4 mm.), of castrated female guinea pig (XXXIV.69) with subcutaneously implanted tablet of estradiol. Sacrificed 130 days after implantation; absorption of 48 μgm. of estradiol daily. The two dark stripes correspond to collagenous tissue with blood vessels. Van Gieson stain. Mag. × 5. Note the difference between Fig. 15 and Figs. 1 and 8, which is recognizable even at low magnification.

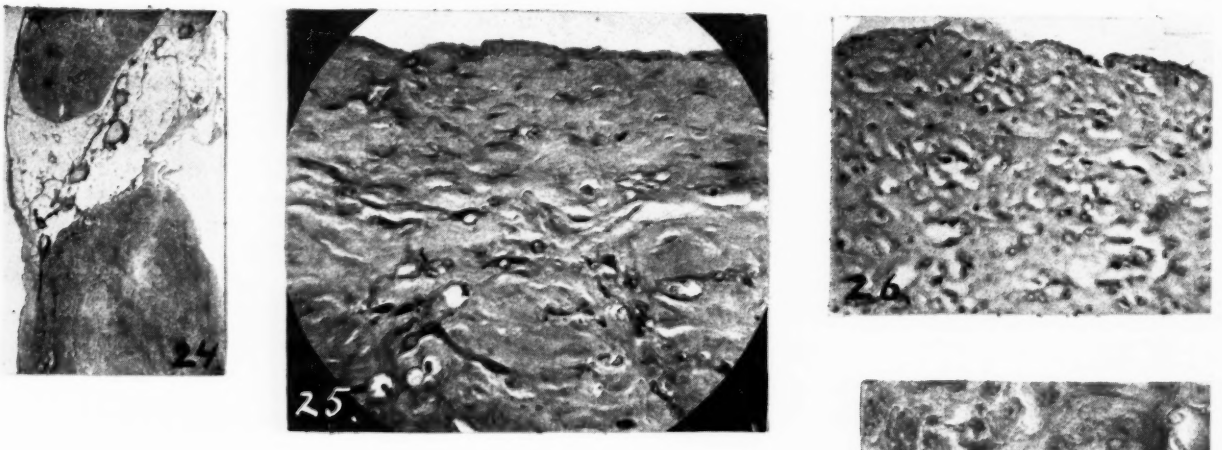
Fig. 16.—Mesometric tumor of class 2 (2 × 3.5 mm.) of the second uterine horn of the same animal as Fig. 15. Mag. × 23. Compare with Figs. 2 and 9.

Fig. 17.—The same tumor as in Fig. 16. Great number of spindle-shaped cells separated one from another by collagenous fibers. Mag. × 200. Compare with Figs. 3, 7, and 10.

Fig. 18.—Mesenteric tumor of class 3 (5 × 8 mm.) of castrated female guinea pig (XXXIV.50) with subcutaneously implanted tablet of estradiol. Sacrificed 130 days after implantation; absorption of 45 μgm. of estradiol per day. Spindle-shaped cells; abundant collagenous fibers between these cells. Van Gieson stain. Mag. × 200. Compare with Fig. 11.

Fig. 19.—Another section from the same mesenteric tumor as in Fig. 18. Greater abundance of collagenous fibers.





FIGS. 20-29

A similar picture of regression was found in a *mesenteric* fibroid (Figs. 24 to 26); it is instructive to compare this with a mesenteric fibroid of an animal that had received estradiol for the same length of time but without the subsequent implantation of progesterone (Figs. 18 and 19).

Far reaching sclerosis without spindle-shaped cells in a tumor of the hilum of the spleen is shown in Figs. 27 and 28. There is also partial necrotization (Fig. 28), and giant cells are present (Fig. 29). A great number of giant cells had been found formerly in experimental abdominal fibroids transplanted into the abdominal cavity of other animals (unpublished work of Lipschütz and Koref).

It follows from the experiments described above that degenerative changes take place in estrogen-induced abdominal fibroids subjected subsequently to the action of progesterone. These changes coincide with those undergone by fibroids after suspension of estrogen or after intra-abdominal transplantation. Three parallel sets of experiments are thus available in which similar degenerative changes occur in estrogen-induced abdominal fibroids. It is noticeable that a similar process of hyalinization is apparently present also in estrogen-induced growing fibroids; near the center of the growing tumor the number of glassy collagenous fibers increases. What characterizes the growing fibroid is presumably not the complete absence of the hyalinizing tendency but the presence of the peripheral zone of fibroblasts, or of cellular proliferation, and the minor amount of collagenous tissue.

DISCUSSION

Our microscopic findings fully corroborate the conclusion drawn in a previous paper, that regression of uterine and other abdominal fibroids produced in the guinea pig by the prolonged administration of estradiol takes place when progesterone is subsequently added and allowed to act simultaneously with the estrogenic or fibromatogenic hormone.

The senior author has emphasized the many analogies that exist between uterine fibroids induced experimentally by estrogens, on the one hand, and uterine fibroids in women on the other (Lipschütz, 3). These analogies refer to localization (subserous, mesometrial), to structure, to concomitance with atypical epithelial growth (the cystic glandular hyperplasia and adenomatous polyps that are present simultaneously with experimental or spontaneous fibroids), and to behavior (regression in absence of estrogens). On the basis of these analogies, although they are not perfect, and on the basis of available clinical data, Lipschütz has drawn the conclusion that the uterine fibromyoma in women is probably due to some endocrine ovarian disturbance that expresses itself in a prolonged follicular phase (3). All this makes it very likely that the preventive and therapeutic antifibromatogenic action of progesterone and other steroids, as demonstrated in our experiments in the guinea pig, would apply also in women.

There is one very important aspect that must not be overlooked in any discussion of the fibromatogenic and antifibromatogenic actions of steroids. Each region or territory of the body reacts in its own way to the proliferative stimulus of the estrogen, and the differential sensitivity of the territories varies according to species (4, 5, 6). One must suppose that similar laws govern also the antiproliferative actions of steroids; there are various observations in favor of such an assumption (5). Consequently, it cannot be inferred that progesterone, which has been revealed to be the most potent antifibromatogenic steroid for the guinea

DESCRIPTION OF FIGURES 20 TO 29

FIG. 20.—Uterine tumor of class 2 (2 × 3 mm.) of castrated female guinea pig (XXXIV.2) with subcutaneously implanted tablets of estradiol and progesterone. Sacrificed 78 days after implantation of estradiol, and 37 days after implantation of progesterone. See diagram of this animal in Fig. 4 of the preceding paper. Van Gieson stain. Mag. × 5. Compare with Figs. 12 and 15.

FIG. 21.—Another section of the same tumor as in Fig. 20. Mag. × 23. Compare with Fig. 16.

FIG. 22.—The same tumor as in Figs. 20 and 21. Hyalinization of peripheral part of the tumor. Mag. × 200. Compare with Figs. 13 and 17.

FIG. 23.—The same tumor as in Figs. 20, 21, and 22. Accumulation of cells; mostly small nuclei. Mag. × 200. Compare with Fig. 14.

FIG. 24.—Large mesenteric tumor of class 3 (5 × 10 mm.) of the same animal as in Figs. 20 to 23. Above—mesenteric node surrounded by adipose tissue with blood vessels; below—part of mesenteric tumor. Van Gieson stain. Mag. × 5.

FIG. 25.—The same tumor as in Fig. 24. Note scarcity of cells and abundant hyalinized collagenous tissue.

FIG. 26.—Another section from the same tumor as in Figs. 24 and 25. Greater abundance of cells with small nuclei. Mag. × 200. Compare with Figs. 18 and 19.

FIG. 27.—Tumor of the hilum of the spleen of class 2 (4 × 4 mm.) of castrated female guinea pig (XXXIV.19) with subcutaneously implanted tablets of estradiol and progesterone. Sacrificed 76 days after implantation of estradiol and 34 days after implantation of progesterone. Absorption of 41 μgm. of estradiol and of 282 μgm. of progesterone per day. The tumor adheres to the intestine and infiltrates its outer muscular coat. It consists mostly of hyalinized collagenous tissue. Van Gieson stain. Mag. × 5.

FIG. 28.—The same tumor as in Fig. 27. Necrotic masses of semilunar shape surrounded by hyalinized tissue. Hematoxylin and eosin stain. Mag. × 23.

FIG. 29.—The same tumor as in Figs. 27 and 28. Two giant cells near necrotic masses. Van Gieson stain. Mag. × 400.

pig, would be the most powerful antifibromatogenic therapeutic steroid in women. It must be determined empirically which of the three available synthetic antifibromatogenic steroids—testosterone, desoxycorticosterone, progesterone—is preferable in the treatment of uterine fibroids in women.

SUMMARY

The microscopic structure of uterine and other abdominal fibroids induced in the guinea pig by the continuous action of alpha-estradiol, and subsequently subjected to the simultaneous action of progesterone, was compared with that of similar fibroids that underwent regression after withdrawal of the estrogen.

The structure of tumors that were still present 34 to 53 days after the beginning of progesterone treatment was similar to that of tumors that shrank after withdrawal of the estrogen.

In both cases the most conspicuous findings were the disappearance of the spindle-shaped cells (fibroblasts) from the periphery of the tumor and the transformation of the tumor into a uniform mass of more or less hyalinized collagenous tissue with small scattered nuclei.

The statement that under the influence of progesterone uterine or other abdominal fibroids undergo structural changes characteristic of regression and similar to those taking place after the withdrawal of estrogen, fully corroborates the conclusion drawn in the previous paper, that fibroids induced by estrogens in the course of several months begin to regress when progesterone is added subsequently and allowed to act simultaneously with the estrogenic hormone.

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Effect of Testosterone Propionate on the Adrenals and on the Incidence of Mammary Cancer in the RIII Strain of Mice

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INTRODUCTION

The hormonal relationship that Cramer and Horning (6, 8, 9) believe to exist between brown degeneration of the adrenals and carcinoma of the mammae in high tumor strain mice has been questioned by other observers, but not definitely disproved. Details may be found in the papers of Dobrovolskaïa-Zavadskaïa and her group (15-17); Lacassagne and Raynaud (25, 26); Blaisdell, Gardner, and Strong (1); Bonser (3); and Kreyberg (23). This degeneration occurs frequently at an early age in female mice of a high tumor strain, according to Cramer (8) and Dobrovolskaïa-Zavadskaïa (17), but is seen also in the tumor-free males of the same strain at a later age. It has been described by Whitehead (31), Martin (28), Howard-Miller (21), Burrows (5), Daughady (11), and Deanesly (12) as seen at the cortico-medullary junction, in the periphery of the medulla, or in the medial part of the zona reticularis. The change significantly begins in the x-zone, which, according to Howard-Miller, is "A transitory zone showing age and sex relationships in mice."

The appearance of brown degeneration around the x-zone in various strains of mice after estrinization as described by Burrows (4, 5), Cramer (6), and Martin (28), and the induction of mammary tumors in similar strains also by estrinization reported by Cramer (10), Bonser and her associates (2), Lacassagne (24), Twombly (30), and Gardner (18) warranted further studies on this apparent relationship.

Burrows (5) states that the effect of castration, pregnancy, and estrogen on the presence or absence of the x-zone suggests that it may be influenced by testosterone or progesterone, and notes that these hormones seem to hasten the involution or absorption that normally occurs in the x-zone of adrenals in female mice. Deanesly points out that castration of the male causes reappearance of the x-zone, converting the male adrenal to the female type. Woolley, Fekete, and Little (32) have observed nodular hyperplasia and carcinoma of the adrenals in ovariectomized mice that show a return of estrogen effect. Martin (28) noted the disappearance of the x-zone in females after testosterone. Thus a definite relationship has been shown to exist between sex hormones and adrenals on the one hand, and the same hormones and certain mammary tumors on the other.

The problem that presented itself, therefore, dealt with the administration of certain hormones to mice with a high incidence of both mammary tumors and adrenal degeneration.

MATERIALS AND METHOD

The experimental data reported here deal with brown degeneration of the adrenals, in relation to mammary carcinoma in female mice of the RIII strain, after the administration of testosterone propionate.[1] These mice were inbred from animals procured from Dr. N. Dobrovolskaïa-Zavadskaïa (13) by Dr. F. C. Wood in May, 1935.

In order to determine approximately the incidence of spontaneous mammary carcinoma in RIII breeding female mice in this laboratory, 486 were observed from the fourth to the eighteenth month of life. For several reasons, including the diminishing incidence after 18 months (14), all animals without tumors were eliminated at that age and classed with those dying before that age as negative. Figures for comparison between untreated and treated animals were sought because various observers have reported incidences ranging between 56 and 79 per cent in untreated mice (15, 24). Of the 486 stock mice observed, 254 developed mammary adenocarcinoma (52.2%). A small number had 2 or more tumors. The earliest tumor to be noted arose at the age of 180 days, the remainder appearing between 200 and 326 days of age. Dobrovolskaïa-Zavadskaïa and Kobozieff (14) reported that tumors appeared in 37.3 per cent of RIII mice 18 months old.

[1] Testosterone propionate for these experiments was generously furnished by Dr. Erwin Schwenk, of the Schering Corporation; Dr. R. J. Floody, of Roche-Organon, Incorporated; and Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, Incorporated.

Observations on the Growth of Tumors in RIII Mice after Injection of Testosterone

Between May 2, 1941 and August 11, 1942, 96 breeding female mice, 5 to 7 months old, were injected subcutaneously every 2 weeks with 0.5 to 1.0 mgm. of testosterone propionate in peanut oil (total of 8 to 9 mgm.). Animals between these ages were chosen because the mammary gland was fully developed. As Lacassagne (24) has shown that mammary tissue atrophies when androgens are administered to female mice soon after birth, it would have been impossible to evaluate the pharmacologic effect of testosterone on a mature precancerous gland unless treatment were begun at maturity. Twelve mice, 6 months old, received cutaneous inunctions over epilated areas every 2 weeks (total 18 mgm.). As the animals aged the injections and inunctions were given every third and finally every fourth week. All were observed until death, which occurred between 8 and 20 months of age.

Table I gives details of the experiment. Spontaneous mammary tumors appeared in 19.4 per cent (21 of 108) of androgen treated female mice; when 24 tumor-free mice that died before the age of 1 year were eliminated the tumor-incidence was 25 per cent (21 tumors in 84 mice). The incidence in untreated animals, as previously mentioned, was 52.2 per cent. In the different series of treated mice the percentage of tumors arising varied between 16.6 and 25.0 per cent depending on dosage, time, and method of administration, and age of animals at death. Mice receiving a larger dose (18 mgm.) in series III, and those treated for a shorter time in series IV (8 months), gave a lower percentage of growths. Nathanson and Andervont (29) reported an incidence reduced to 30 per cent after testosterone injections. Similar results were reported by E. E. Jones (22) and also by A. A. Loeser (27).

None of the mice subjected to testosterone treatment bore any litters.

Tumors arising in injected mice were not influenced or inhibited by further treatment with testosterone. Multiple tumors did not appear in treated mice, but occurred frequently in the untreated controls. The time elapsing between the first treatment and the discovery of tumors varied between 28 and 372 days. It is probable that in 5 mice minute tumors were already present when treatment was started, since they became manifest within 4 weeks after the first injection of 0.5 mgm. of testosterone propionate. It might have been better, therefore, if treatment had been started at 4 months of age instead of 6. In addition to these early tumors 4 appeared after treatment had been discontinued for 56 days or more.

Table I: Tumors in Testosteronized Female Mice of the RIII Strain

Series	Number of mice	Age at beginning of treatment	Amount of testosterone	Duration of treatment	Number of tumors	Per cent
I Box 1	24	7 mos.	8.7 mgm.			
Box 2			16 injections	14 mos.	6	25.0
II Box 3	24	5½ "	9.7 mgm.	15.6 "	5	20.8
Box 4			18 injections			
III Box 1	12	6 "	18 mgm.			
			18 inunctions	15 "	2	16.6
IV Box 1	48	5½-6 "	9 mgm.	8 "	8	16.6
Box 2			9 injections			
Box 3						
Box 4						

In 5 mice tumors appeared within 28 days after first treatment.
In 1 mouse tumor " " 42 " " " "
" " " " " " 56 " " termination of "
" " " " " " 372 " " first "
24 mice without tumors died between the 8th and 12th months.

The various types of mammary carcinoma arising in androgen treated mice differed in no way from those in the untreated. The tumors ranged between adenocarcinoma of the simple or medullary forms, cystic degenerative types, and tumors with extensive hemorrhagic areas.

Observations on Brown Degeneration in the Adrenals of Normal and Testosteronized RIII Mice

Histologic studies of both adrenals of 135 mice were made. Some of the glands were stained with osmic acid vapor as described by Cramer (7), others with Sudan IV and hematoxylin or eosin and hematoxylin. The amount of brown degeneration found was classi-

fied approximately between 0 and 4+ (Table II). The greatest number of adrenals with degeneration appeared in untreated mice (92.8%) with tumors; in treated mice with tumors the number was somewhat smaller (85.7%). In certain treated mice tumors continued to grow and brown degeneration occurred despite the injections. On the other hand, a still smaller proportion (80.9%) of degenerated adrenals in untreated tumor-free mice, and considerably fewer (56.6%) in tumor-free androgen-treated female mice according to Burrows (5), Martin (28), and others seems to effect a rapid involution of the x-zone, and in our series of females the same hormone produced a significant reduction of adrenal degeneration in the same region. Jones (22) and others, as previously mentioned, reported a reduced incidence of tumors in mice after testosterone injections. Since both brown degeneration and tumor incidence are reduced by testosterone, it seems not unreasonable to assume that more than a casual relationship exists between the two.

TABLE II: BROWN DEGENERATION IN THE ADRENALS OF FEMALE MICE OF THE RIII STRAIN

	Number of females	Brown degeneration					Per cent
		0	1+	2+	3+	4+	
Untreated controls; no tumors	36	4	6	14	8	4	80.9
Treated: Testosterone propionate; no tumors	50	22	13	10	3	2	56.6
Untreated controls; tumors	28	2	8	7	2	9	92.8
Treated: Testosterone propionate; tumors	21	3	12	2	1	3	85.7

supplements the observation that in testosterone-sensitive mice a parallel reduction in tumor growth and brown degeneration occurs (Table III).

In other words, animals developing tumors in spite of the treatment had almost as much adrenal degeneration as untreated tumor-bearing mice. Those subjected to treatment and remaining free of tumors exhibited much less brown degeneration. A parallel reduction of tumor incidence and adrenal degeneration after testosterone, therefore, appears in this strain.

TABLE III: BROWN DEGENERATION AND TUMOR INCIDENCE IN RIII MICE

	Tumors, per cent	Brown degeneration, per cent
Untreated (36 mice)	0	80.9
Treated (50 ")	0	56.6
Untreated (28 ")	52.2	92.8
Treated (21 ")	19.4	85.7

Corresponding drop in tumor incidence and brown degeneration in treated mice.

In androgen-treated mice with tumors the adrenals, as well as the ovaries and adnexa, were atrophied, while in a similarly treated group of mice without tumors the adrenals were notably enlarged, but the ovaries and uterine horns were atrophied.

DISCUSSION

Since the hormones of the adrenal cortex are not sex-specific the possibility of assigning androgenicity or estrogenicity to special cells or layers is problematic. The x-zone has been described as a transitory structure, whose morphologic status is influenced by the gonadal or hormonal status of the host. Testosterone,

Apart from this relationship the effect of testosterone as a possible inhibitor of mammary gland proliferation may be considered. In 1940 reports were published (19) describing the inhibiting effect of androgens on the epithelium of benign fibroadenomas of the rat, and (20) the considerably reduced occurrence of the same tumors in male castrates after testosterone was administered. On the basis of these observations and of those recorded in the present paper the suggestion is advanced that testosterone in controlled dosage be used under careful supervision in women with a family history of mammary cancer as a prophylactic measure shortly before the cancer age. The amount of testosterone given orally or by injection may be about the same as that now used for the menopausal syndrome, or other gynecological disturbances, or in certain conditions of the mammary gland such as mastodynia.

CONCLUSION

1. In testosteronized female mice of the RIII strain the incidence of mammary carcinoma was reduced from 52.2 to 19.4 per cent.

2. In the same series brown degeneration of the adrenals was reduced from 80.9 per cent in controls to 56.6 per cent in tumor-free treated mice.

3. In testosteronized mice exhibiting tumors brown degeneration of the adrenals was not diminished (85.7%).

4. A lowered tumor incidence and brown degeneration occurred in testosteronized female mice of the RIII strain. The reduction of a stimulating factor in the mammary gland, and of an inhibiting factor in the adrenal gland, appears to represent more than a casual

relationship, established, perhaps, by the effects of testosterone.

5. The lowering of tumor frequency may presumably be due to reduction of the growth activity of the mammary gland epithelium, when associated in testosteronized mice with adrenal changes.

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The Prothrombin Concentration in the Plasma of Normal and Leukemic Rats*

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It was noted in a previous investigation of a transmissible lymphoid leukemia of the rat (2) that animals in the late stage of the disease frequently show active bleeding from the nose and conjunctivae. Autopsy of these animals reveals enormous enlargement of the liver due to leukemic infiltration and extensive hemorrhages in the lungs, liver, testes, subcutaneous tissue, and in the abdominal and femoral muscles. Other rats dying with the disease in an equally fulminant form show no evidence at autopsy of a hemorrhagic diathesis and relatively little leukemic infiltration of the liver.

A series of experiments has been carried out to determine whether variations in plasma prothrombin level might be the controlling factor in the difference in hemorrhagic tendency. Quick's method (3, 4) for prothrombin time determination was utilized in this study.

METHODS

Young rats of the Wistar strain were injected intraperitoneally with 0.2 cc. of a suspension of leukemic cells. Between the seventh and ninth day following injection there appeared clinical manifestations of the disease: namely, generalized lymphadenopathy, hemorrhages from the nose and conjunctivae, and elevation of the white blood count. When the disease had reached an advanced stage, 2.7 cc. of blood were drawn from the heart into a syringe containing 0.3 cc. of 0.1 *M* sodium oxalate solution. The red blood cells were removed by centrifugation, and 0.1 cc. of the supernatant clear plasma was placed in a test tube. To this plasma were added 0.1 cc. thromboplastin solution and 0.1 cc. of 0.025 *M* calcium chloride solution. The tube was gently shaken, placed in a water bath at 38° C., and the clotting time recorded with a stop watch.

The thromboplastin solution was prepared by the emulsification of 0.3 gm. of dehydrated rabbit brain in 4.9 cc. physiological salt solution and 0.1 cc. of 0.1 *M* sodium oxalate. The mixture was incubated at 45° C. for 10 minutes and then centrifuged for 3 minutes to remove the larger particles. The milky supernatant fluid was used in the majority of the tests, although a commercial product was substituted in a few instances.

Experiment 1.—The prothrombin time of plasma from 12 rats with well established leukemia and 20 normal rats of the same age and strain was estimated by Quick's method. The average prothrombin time for the leukemic plasma was 31 seconds, while that for the normal plasma was 25.8 seconds. The difference was not considered significant.

Since all the leukemic rats of the group above showed extensive hemorrhages and prolonged clotting time, it seemed possible that the hemorrhagic diathesis was caused by a reduction in thromboplastin. Actually there is some evidence of thromboplastin deficiency, since the blood platelets are usually reduced in the terminal stage of the disease. In view of the notable infiltration of the liver and bone marrow by the tumor cells, it is surprising, however, that the animals should appear to have almost the normal amount of prothrombin. The prothrombin concentration might be considerably below normal and yet enough above a critical level to yield a relatively normal prothrombin time. A phenomenon of this type would be demonstrable by dilution of the plasma.

Experiment 2.—In the course of a year, an estimate was made of the prothrombin time of whole plasma and of plasma diluted with physiological saline from 86 rats with advanced leukemia. The animals were divided into two groups: the first included those without gross hemorrhages; and the second, animals with extensive infiltration of the liver and multiple hemorrhages. As a control, the same test was made on samples of plasma from 48 normal rats of the same strain, age, and average weight. Tests were also made on the plasma of 14 rats with large lymphosarcomas that had metastasized to regional lymph nodes. These tumors developed as a result of inoculation of leukemic cells subcutaneously in the groin, a method that produces local tumors without generalized leukemia, liver damage, or hemorrhagic tendency. The percentage of prothrombin was calculated as follows:

$$\frac{\text{Normal animal prothrombin time}}{\text{Test animal prothrombin time}} \times 100.$$

* This investigation was aided by a fund for leukemia studies, contributed anonymously.

TABLE I: RESULTS OF THE QUICK TEST FOR PROTHROMBIN APPLIED TO THE PLASMA OF NORMAL AND LEUKEMIC RATS

Source of Plasma	Number of tests	Average prothrombin in plasma, percent		
		Undiluted	Diluted 1:1	Diluted 1:2
Normal rats	48	100.0	68.4	57.7
Rats with lymphosarcoma	14	92.0	63.4	59.0
Leukemic rats. No hemorrhage	37	83.8	42.6	24.7
Leukemic rats. Liver involvement. Extensive hemorrhage	49	76.4	29.2	18.5

The average figures for the four groups are presented in Table I and are shown graphically in Fig. 1.

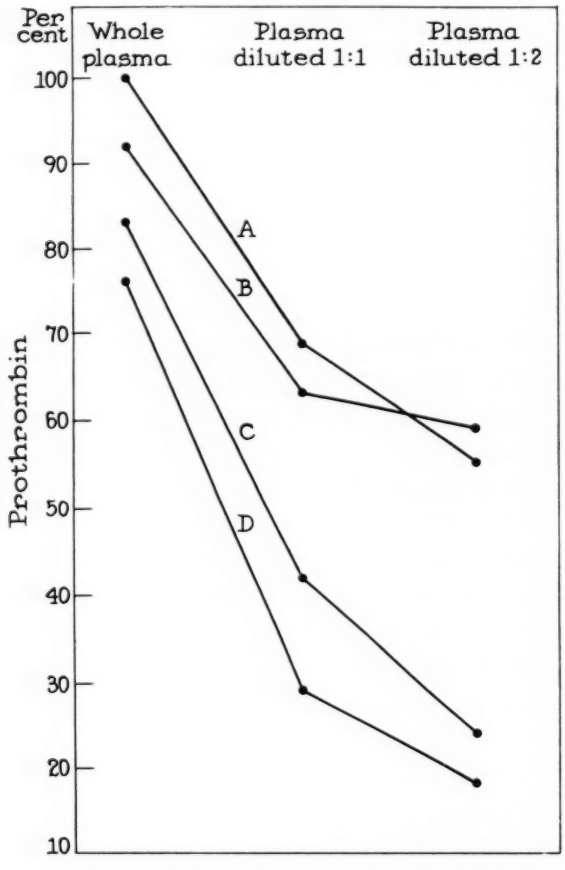

FIG. 1.—Effect of the dilution of the plasma on prothrombin time. (A) Plasma from normal animal. (B) Plasma from rats with lymphosarcoma. (C) Plasma from rats with acute leukemia without extensive hemorrhages or liver damage. (D) Plasma from rats showing pronounced hemorrhagic condition and liver damage.

The average prothrombin time for rats with liver damage and extensive hemorrhages does not include the figures for 10 animals whose plasma failed to clot under 180 seconds when diluted 1:1. The prothrombin time for the plasma of several of these animals ranged from 240 to 600 seconds.

SUMMARY

Whole plasma from leukemic rats with pronounced liver involvement and hemorrhagic tendency shows little difference in prothrombin time from normal plasma. A large deviation from normal is evident when the leukemic plasma is diluted 1:1 and 1:2 with saline and compared with normal plasma similarly diluted. The results indicate that a plasma prothrombin deficiency exists in a transmissible rat leukemia associated with extensive leukemic infiltration of the liver and spontaneous hemorrhages. The deficiency is not demonstrated in whole plasma by means of the Quick test, but is clearly apparent when the plasma is diluted.

The present observations are in accordance with those of Kark and Lozner (1), who found that dilution of human plasma may bring out evidences of prothrombin deficiency not demonstrable when tests are made on whole plasma.

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A Study of *d*-Amino Acid Oxidase, Uricase, and Choline Oxidase in the Livers and in Isolated Liver Cell Nuclei of Rats Bearing Transplanted Tumors*

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Since the report of Brahn (1) that very low catalase values are found in the livers of human beings dead of various forms of cancer, many papers have appeared on the effect of a growing tumor upon enzyme systems in liver tissue. Greenstein (4) has shown that in rats bearing transplanted hepatoma 31 or Jensen sarcoma, or in mice bearing various transplanted tumors, the activity of the liver catalase is notably decreased, but the activity of xanthine dehydrogenase and of acid and alkaline phosphatase is normal. Greenstein also has shown that arginase is decreased in the livers of tumor-bearing rats but not in the livers of tumor-bearing mice. Shack (8) has reported that the *d*-amino acid oxidase is not significantly low in the livers of rats bearing transplanted hepatoma 31, although the concentration of riboflavin has been found low in the livers of rats bearing transplanted tumors. However, no attempt has been made to determine the apoenzyme and coenzyme of *d*-amino acid oxidase separately.

In this work we have investigated apoenzyme and coenzyme of *d*-amino acid oxidase separately and have found that the presence of subcutaneous transplants of hepatoma 31 causes significant lowering of both *d*-amino acid oxidase apoenzyme and coenzyme in whole liver tissue of rats bearing this tumor and in nuclei isolated from the liver cells. We have also investigated the enzymes uricase and choline oxidase in the livers of rats bearing transplanted tumors and in nuclei isolated from the cells of these livers. No work on the latter two enzymes has previously been reported in this connection.

EXPERIMENTAL

We have used both Osborne-Mendel rats bearing hepatoma 31 transplants and Wistar rats bearing carcinosarcoma 256 transplants in our experiments. All the rats were maintained on a fox chow diet fed *ad libitum*.

* This investigation was made possible by a grant from The International Cancer Research Foundation.

The enzyme systems have been studied both in the whole liver tissue and in the isolated nuclei of the liver cells.

Preparation of whole tissue suspension.—All the rats were killed by decapitation and the blood was drained from them as completely as possible. The blood remaining on the livers was rinsed off with a small amount of saline. The livers were cut into small pieces and were ground with 0.9 per cent saline in a glass homogenizer into a homogeneous, cell-free suspension. The suspension was freed from fiber by passing through cheese cloth. The time from killing the rats to starting the oxygen consumption measurements was limited to 30 minutes. For dry weight determinations, 1 cc. of the suspension was dried in a weighed crucible to constant weight at 105° C. in an electric oven.

Preparation of isolated cell nuclei from rat livers.—The livers were removed immediately from the rats after decapitation and frozen. The nuclei of the liver cells were prepared according to the method of Dounce (3).

Preparation of coenzyme of d-amino acid oxidase.—The coenzyme of *d*-amino acid oxidase was prepared from bakers' yeast according to the method of Warburg and Christian (10).

Determination of d-amino acid oxidase apoenzyme.—The *d*-amino acid oxidase was determined by oxygen consumption measurements, using a Warburg apparatus. The method of Klein (5) was followed except that air was used instead of pure oxygen. The total volume of the solutions in the vessels was 2 cc. in all experiments. The oxygen uptake of the whole liver tissue or of the isolated liver cell nuclei caused by the oxidation of *dl*-alanine was recorded at 10 or 15 minute intervals during a period of 1 hour. *dl*-Alanine was used as substrate for all the determinations, since the oxidation of *l*-alanine under the conditions of the experiment has been shown by Krebs to be negligible (6). The substrate and the coenzyme of *d*-amino acid

oxidase, if any was added, were both dissolved in pyrophosphate buffer at pH 8.3. The controls without the substrate were carried out under the same conditions at the same time. In order to study the effect of coenzyme on the oxidation of *dl*-alanine in the whole liver tissue and in isolated liver cell nulcei, a sufficient amount of the above preparation was added to insure maximum enzyme activity.

Determination of uricase.—The uricase activity was determined by the method of Davidson (2) as modified by Elvehjem and his co-workers (9). The determinations were carried out in a Warburg apparatus at 37° C., and the oxygen uptake caused by the oxidation of uric acid was recorded at 10 or 15 minute intervals for 1 hour. The uricase activity was expressed as the oxygen uptake per hour per mgm. of dried tissue.

In order to confirm the presence of uricase, the poisoning effect of KCN was also tested.

Since zinc ions, thought by some to be necessary for uricase action (9), might be removed during the preparation of nuclei, zinc ions were added to the suspensions of nuclei in concentrations employed by Wachtel, Hove, Elvehjem, and Hart (9), but no activating effect was found.

Determination of choline oxidase.—The choline oxidase was determined by the method of Mann and Quastel (7). The oxygen uptake caused by the oxidation of choline hydrochloride by the whole liver tissue or by the isolated liver cell nuclei was measured in a Warburg apparatus at 10 or 15 minute intervals for a period of 1 hour, and the activity was expressed as the oxygen uptake per hour per mgm. of dried tissue.

Number of determinations carried out for each average value of enzyme activity reported.—A total of 5 to 6 animals was used in each experiment, which was performed in duplicate or triplicate. Any results that were not in agreement within 3 per cent were discarded. The liver and the tumor cell nuclei were prepared from 100 gm. of liver or tumor. Three to four experiments were done in each case.

RESULTS

d-Amino acid oxidase apoenzyme in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—It has been found that *d*-amino acid oxidase apoenzyme is low in the whole liver tissue of Osborne-Mendel rats bearing subcutaneous transplants of hepatoma 31. The oxygen uptake caused by the oxidation of *dl*-alanine in the livers of Osborne-Mendel rats bearing hepatoma 31 transplants varies from 0.16 to 0.82 cu. mm. per hour per mgm. of dried tissue, depending upon the size of the transplanted tumor, with an average value of 0.51 cu. mm. The larger the transplanted tumor, the less is the concentration of the enzyme in the livers of the tumor-bearing rats. The average value of 0.51 cu. mm. is only 22 per cent of the average value for normal livers of this strain of rat.

On addition of the coenzyme of *d*-amino acid oxidase to a whole liver suspension from an Osborne-Mendel rat bearing a large subcutaneous transplant of hepatoma 31, the oxygen uptake attributable to the oxidation of *dl*-alanine rose from 0.16 to 0.75 cu. mm. per hour per mgm. of dried tissue, which is still only 32 per cent of the value of normal liver. Similar results were obtained with several rats bearing transplants of hepatoma 31 of about the same size.

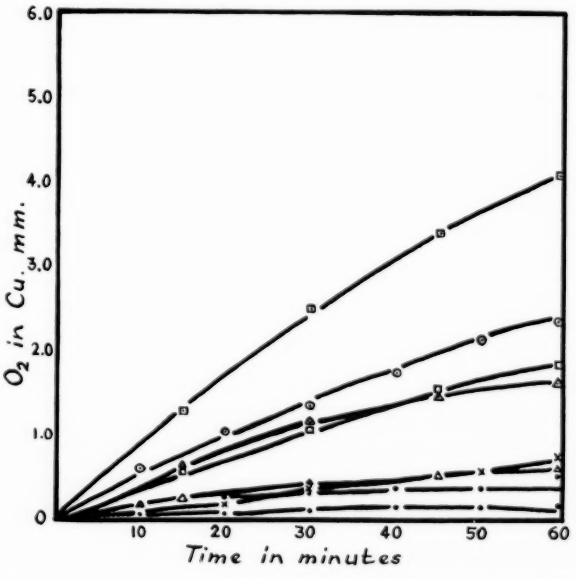

FIG. 1.—*d*-Amino acid oxidase in whole liver tissue and in isolated liver cell nuclei of Osborne-Mendel rats bearing transplanted hepatoma 31, and of normal Osborne-Mendel rats. Solid circle = Whole liver tissue of tumor-bearing rats (upper curve represents the average values; lower curve, the values obtained from rats in serious condition). Cross = Whole liver tissue of tumor-bearing rats plus coenzyme of *d*-amino acid oxidase. Triangle = Liver cell nuclei of tumor-bearing rats. Square = Liver cell nuclei of tumor-bearing rats plus coenzyme of *d*-amino acid oxidase. Circle with dot = Whole liver tissue of normal rats. Triangle with dot = Liver cell nuclei of normal rats. Square with dot = Liver cell nuclei of normal rats plus coenzyme of *d*-amino acid oxidase.

The average value for oxygen uptake attributable to the oxidation of *dl*-alanine in whole liver suspensions from Wistar rats bearing subcutaneous transplants of Walker carcinosarcoma 256, was 1.01 cu. mm. per hour per mgm. of dried tissue, which is about 41 per cent of the average value of normal liver. This value was increased to 1.21 cu. mm. by the addition of the coenzyme of *d*-amino acid oxidase.

In nuclei isolated from the liver cells of Osborne-Mendel rats bearing transplanted hepatoma 31, the increase in oxygen uptake caused by the oxidation of *dl*-alanine without added coenzyme was nearly the same as that of the corresponding whole liver tissue, as shown in Fig. 1. However, the oxygen uptake for

the isolated nuclei rose from 0.61 to 1.84 cu. mm. per hour per mgm. of dried tissue when coenzyme was added, but this value is still only 45 per cent of the value of normal liver cell nuclei under the same conditions.

In the nuclei isolated from the livers of Wistar rats bearing Walker carcinosarcoma 256 transplants, the increase in oxygen uptake caused by the oxidation of *dl*-alanine was very slight. However, it increased from 0 to 0.54 cu. mm. per hour per mgm. of dried tissue after the addition of the coenzyme of *d*-amino acid oxidase, a value which is 49 per cent of that of

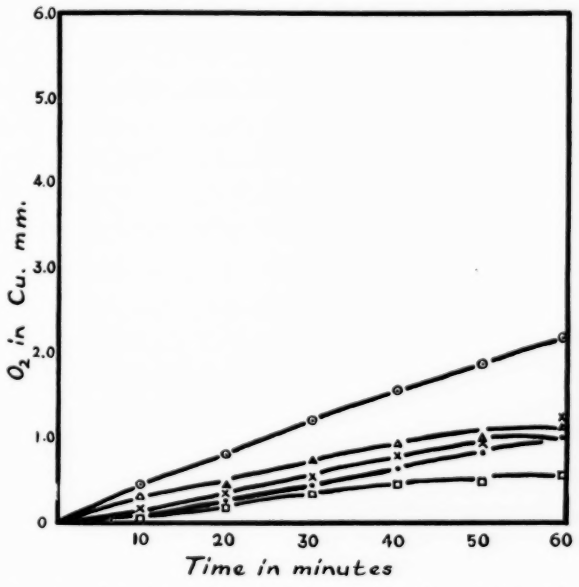

FIG. 2.—*d*-Amino acid oxidase in whole liver tissue and in isolated liver cell nuclei of Wistar rats bearing transplanted Walker carcinosarcoma 256, and of normal Wistar rats. Circle with dot = Whole liver tissue of normal rats. Triangle = Liver cell nuclei of normal rats. Solid circle = Whole liver tissue of tumor-bearing rats. Cross = Whole liver tissue of tumor-bearing rats plus coenzyme of *d*-amino acid oxidase. Square = Liver cell nuclei of tumor-bearing rats plus coenzyme of *d*-amino acid oxidase.

the normal liver cell nuclei. The results of these determinations are shown in Fig. 2.

Uricase in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—The whole liver tissue and the isolated nuclei of the liver cells of Osborne-Mendel rats bearing transplanted hepatoma 31 contained a lower concentration of uricase than that of normal livers and nuclei from normal liver cells. The oxygen consumption caused by the oxidation of uric acid in whole liver tissue of Osborne-Mendel rats bearing hepatoma 31 transplants varied from 1.26 to 1.66 cu. mm. per hour per mgm. of dried tissue, with an average value of about 1.46 cu. mm. This value is 51 per cent of the average value for normal livers. The average value for the oxygen consumption caused by the oxidation of uric acid in the isolated nuclei of the liver cells was 1.93 cu. mm. per hour per mgm. of dried tissue, which is about 46 per cent of the value of normal liver cell nuclei. The results of these determinations are shown in Fig. 3.

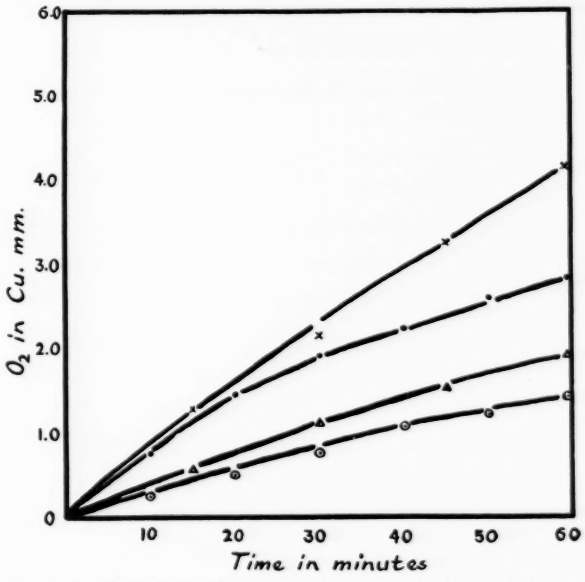

FIG. 3.—Uricase in whole liver tissue and in isolated liver cell nuclei of Osborne-Mendel rats bearing transplanted hepatoma 31, and of normal Osborne-Mendel rats. Circle with dot = Whole liver tissue of tumor-bearing rats. Triangle = Liver cell nuclei of tumor-bearing rats. Solid circle = Whole liver tissue of normal rats. Cross = Liver cell nuclei of normal rats.

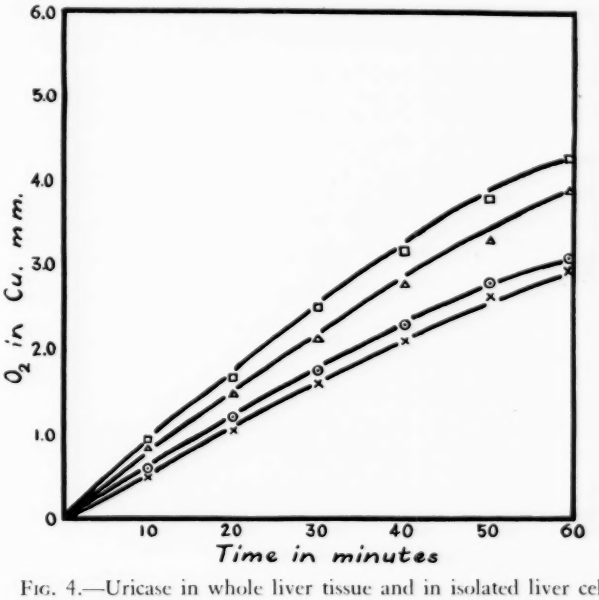

FIG. 4.—Uricase in whole liver tissue and in isolated liver cell nuclei of Wistar rats bearing transplanted Walker carcinosarcoma 256, and of normal Wistar rats. Cross = Whole liver tissue of tumor-bearing rats. Square = Liver cell nuclei of tumor-bearing rats. Circle with dot = Whole liver tissue of normal rats. Triangle = Liver cell nuclei of normal rats.

The livers of Wistar rats bearing Walker carcinosarcoma 256 transplants and nuclei isolated from them contained almost the same concentration of uricase as that of normal livers and their nuclei, as shown in Fig. 4. The oxygen uptake caused by the oxidation

of uric acid in whole liver tissue of Walker tumor-bearing rats was 2.90 cu. mm. per hour per mgm. of dried tissue, and that of nuclei isolated from these livers 4.28 cu. mm.

Choline oxidase in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—The average value for the oxygen uptake of whole liver tissue of Osborne-Mendel rats bearing hepatoma 31 transplants caused by the oxidation of choline was 1.67 cu. mm. per hour per mgm. of dried tissue, which is 69 per cent of the value of normal livers. Choline oxidase in whole liver tissue of Wistar rats bearing carcinosarcoma 256 transplants was almost the same as that of normal Wistar rat liver. The oxygen uptake caused by the oxidation of choline in such livers was 1.81 cu. mm. There was no detectable amount of choline oxidase in the nuclei isolated from the livers of rats bearing transplanted tumors, but neither was there any in normal rat liver cell nuclei. The results of the determinations of choline oxidase are shown in Fig. 5.

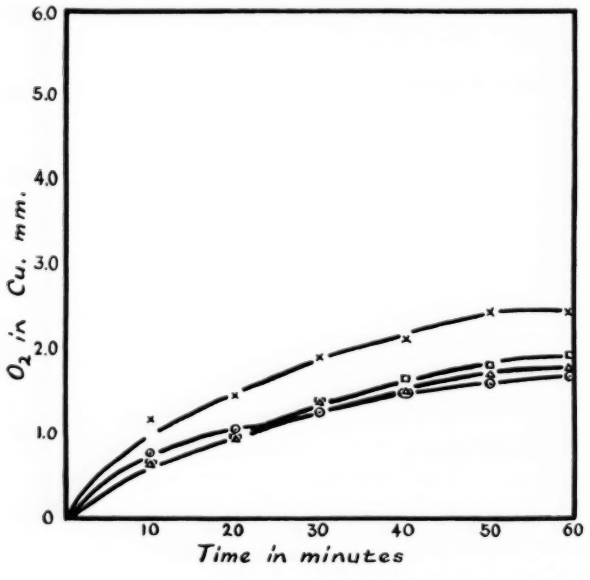

FIG. 5.—Choline oxidase in whole liver tissue of normal and tumor-bearing rats. Circle with dot = Whole liver tissue of tumor-bearing Osborne-Mendel rats. Cross = Whole liver tissue of normal Osborne-Mendel rats. Square = Whole liver tissue of tumor-bearing Wistar rats. Triangle = Whole liver tissue of normal Wistar rats.

DISCUSSION

We have shown that a tumor of liver origin (hepatoma 31) can influence the liver by depleting an apoenzyme more than its coenzyme, since the addition of coenzyme of *d*-amino acid oxidase to the livers of rats bearing subcutaneous transplants of hepatoma 31, and to nuclei isolated from them, does not increase the oxidation of *dl*-alanine to the normal value. Therefore the tumor certainly causes depletion of the apoenzyme of *d*-amino acid oxidase, although the coenzyme also appears to be lowered somewhat. Shack (8), as has been mentioned previously, already had shown that the *d*-amino acid oxidase content of livers of rats bearing subcutaneous transplants of hepatoma 31 is low, but he did not investigate the apoenzyme and coenzyme separately.

From the fact that there is no inhibitor for liver catalase in tumor, or in the livers of tumor-bearing animals, and the fact that hemoglobin as well as liver catalase is low in animals bearing large tumors, Greenstein (11) has concluded that the lowering of these two substances in tumor-bearing animals is caused by an inability of the animal to synthesize the porphyrin ring system present in the prosthetic groups of both hemoglobin and catalase. However, the work reported in this paper indicates a likelihood that at least part of the difficulty lies in the inability of the animal to synthesize the protein components. Tumors in rapid growth might hinder synthesis of the protein components of enzymes by appropriating amino acids, although this is a matter of speculation at the present time.

The Walker carcinosarcoma 256 of Wistar rats is not of liver origin, and it is of considerable interest that this tumor does not appear to influence *d*-amino acid oxidase, uricase, or choline oxidase in the livers of animals bearing it.

SUMMARY

1. An investigation has been made of the concentrations of *d*-amino acid oxidase, uricase, and choline oxidase in livers of rats bearing transplants of hepatoma 31 and carcinosarcoma 256, and in nuclei isolated from the liver cells.

2. A tumor of liver origin (hepatoma 31) caused depletion of the apoenzyme of *d*-amino acid oxidase more than its coenzyme in the livers of animals bearing this tumor. Hepatoma 31 also caused lowering of uricase and choline oxidase in the livers of tumor-bearing animals.

3. Walker rat carcinosarcoma 256 did not cause appreciable lowering of *d*-amino acid oxidase, uricase, or choline oxidase in the livers of animals bearing this tumor.

4. It has been found that hepatoma 31 also lowered the activity of *d*-amino acid oxidase and uricase in isolated nuclei of liver cells of rats bearing this tumor.

5. No choline oxidase activity has been detected in the isolated liver nuclei of rats bearing transplanted tumors. This is true also of nuclei isolated from cells of normal rat liver.

I wish to express my appreciation to Professor W. R. Bloor and Dr. A. L. Dounce for their advice and encouragement throughout the course of this work.

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The *d*-Amino Acid Oxidase, Uricase, and Choline Oxidase in Two Transplanted Rat Tumors and in Isolated Nuclei of Tumor Cells*

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In order to determine whether or not changes in enzymatic activity specifically accompany neoplasia, and to what extent such changes may occur, many enzymes have been investigated by Greenstein (2) in both normal and neoplastic tissues. Shack (3) has reported that *d*-amino acid oxidase is low in rat hepatoma 31, and suggested that the lowering of the enzyme may be owing to a deficiency in protein component as well as in the flavin prosthetic group of the enzyme, but no attempt has been made to determine the apoenzyme and coenzyme of *d*-amino acid oxidase separately. Furthermore, very little work has been done on the change in distribution of enzymes between the nuclei and cytoplasm of tumor tissue, owing to lack of availability of satisfactory preparations of tumor cell nuclei.

In this work both the apoenzyme and coenzyme of *d*-amino acid oxidase have been studied in whole tumor tissue and in isolated nuclei of tumor cells. A study has been made, also, of the enzymes uricase and choline oxidase, two enzymes hitherto not investigated in tumor tissue.

EXPERIMENTAL

Types of tumors investigated.—In this work, Osborne-Mendel rats bearing subcutaneous transplants of hepatoma 31, and Wistar rats bearing subcutaneous transplants of Walker carcinosarcoma 256 have been used. As has been emphasized by Greenstein (2) in his recent review article, enzyme systems in the liver, and in tumors of different origin, may vary considerably from strain to strain.

Preparation of whole tumor tissue suspension.—Whole tissue suspensions were prepared as described in the preceding paper.

Preparation of isolated cell nuclei from hepatoma 31.—Nuclei of hepatoma 31 have been prepared by a modification of the method employed for preparing nuclei of normal rat liver cells at pH 6.0 to 6.2 (1). This modification has been worked out by Dounce, but has not yet been published, since it is not at the present time entirely satisfactory. The principal objection is that nucleic acid appears to be extracted from the tumor nuclei during the process of isolation, and that, therefore, protein and enzymes also may be extracted to an unknown extent. On this account, results reported here for enzymes in hepatoma 31 nuclei must at the present time be considered as tentative.

Preparation of coenzyme of d-amino acid oxidase.—The coenzyme of *d*-amino acid oxidase was prepared from baker's yeast as described in the preceding paper.

Determination of d-amino acid oxidase apoenzyme, uricase, and choline oxidase.—These 3 enzymes were determined as described in the preceding paper.

Number of determinations carried out for each average value reported.—Whenever an average value for the activity of an enzyme in whole tissue is reported in this paper, 5 or 6 analyses have been carried out, each on tissue from a different animal, and each performed in duplicate or triplicate, and the results have been averaged.

In the case of nuclei, analyses were made in duplicate or triplicate on at least 3 different preparations of nuclei. In making each preparation, 100 gm. of tissue was used, which was collected from 10 to 15 animals in case of liver, and 3 to 5 animals in case of tumor.

RESULTS

d-Amino acid oxidase apoenzyme in transplanted rat tumors and in nuclei isolated from tumor cells.—The oxygen uptake of the suspensions of hepatoma 31 caused by the oxidation of *dl*-alanine was 0.07 cu. mm. per hour per mgm. of dried tissue, which is only 3 per cent of the average value of the normal liver. After the coenzyme of *d*-amino acid oxidase had been

* This investigation was made possible by a grant from The International Cancer Research Foundation.

added, the oxygen uptake rose to 0.27 cu. mm., which is 12 per cent of the average value of normal liver. The nuclei isolated from hepatoma 31 showed the same results as the whole tumor tissue.

The transplanted Walker carcinosarcoma 256 showed no activity of *d*-amino acid oxidase. Addition of coenzyme of *d*-amino acid oxidase caused an increase in oxygen uptake of only 0.16 cu. mm., an insignificant amount. Nuclei of Walker carcinosarcoma 256 are not yet available in a state where such enzymes and proteins are not denatured, so that they could not be studied. The results of these determinations are shown in Fig. 1.

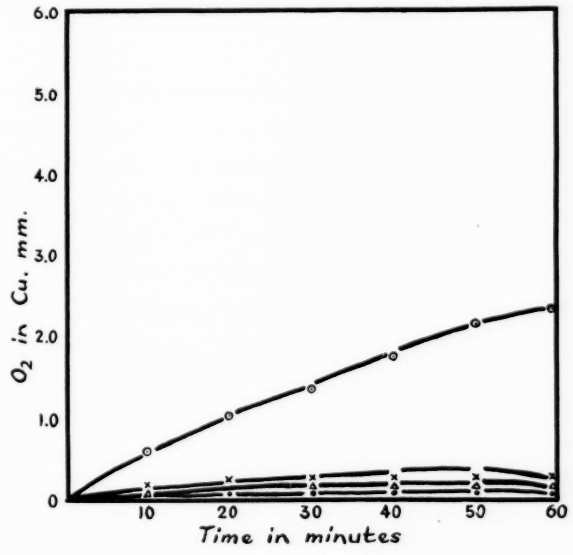

FIG. 1.—*d*-Amino acid oxidase in transplanted hepatoma 31, normal whole rat liver tissue, and Walker carcinosarcoma 256. Circle with dot = Normal whole rat liver tissue. Solid circle = Whole transplanted hepatoma 31 tissue. Cross = Whole transplanted hepatoma 31 tissue plus coenzyme of *d*-amino acid oxidase. Triangle = Walker carcinosarcoma 256 transplants plus coenzyme of *d*-amino acid oxidase.

Uricase in transplanted rat tumors and in nuclei isolated from the tumor cells.—The transplants of hepatoma 31 and their nuclei contained a very low concentration of uricase, as shown by the oxygen consumption in the presence of uric acid. Both hepatoma 31 transplants and the nuclei isolated from them showed an increase in oxygen consumption in the presence of uric acid of about 0.15 cu. mm. per hour per mgm. of dried tissue. The results of uricase determinations are shown in Fig. 2.

Transplants of Walker carcinosarcoma 256 showed no uricase activity.

Choline oxidase in transplanted rat tumors and in nuclei isolated from tumor cells.—Transplants of hepatoma 31 and Walker carcinosarcoma 256, and the nuclei of hepatoma 31, showed no choline oxidase activity.

DISCUSSION

In a recent review article on tumor enzymology Greenstein (2) has shown that transplants of hepatoma 31 generally show less enzyme activity than the primary hepatoma, which still contains some liver cells with enzyme activity nearer to normal than that of the tumor cells. Alkaline phosphatase is an exception, since this is very high in hepatoma 31. In our work we have found very low enzymatic activity of the three enzymes studied in the transplants of hepatoma 31, although these enzymes are characteristically present in normal liver tissue. The *d*-amino acid oxidase

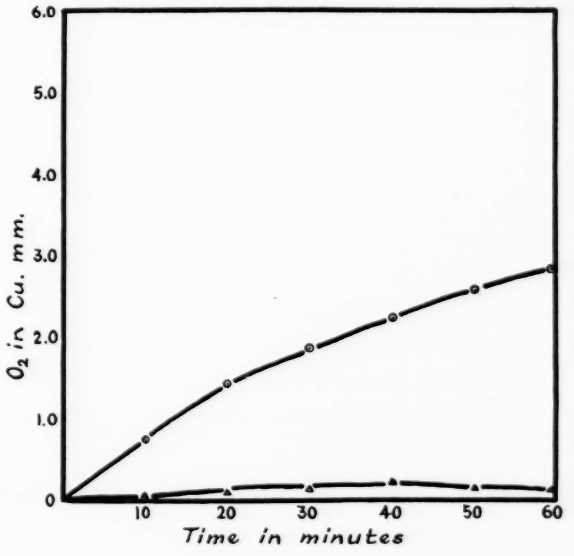

FIG. 2.—Uricase in transplanted hepatoma 31 and in normal whole rat liver tissue. Circle with dot = Normal whole rat liver tissue. Triangle = Whole transplanted hepatoma 31 tissue.

apoenzyme and uricase are greatly decreased in the transplants of hepatoma 31, while choline oxidase is completely lacking in activity in the transplants. Addition of the coenzyme of *d*-amino acid oxidase did not increase the oxidation of *dl*-alanine to any considerable extent, which indicates, as Shack (3) suggested, that the lowering of *d*-amino acid oxidase activity in hepatoma 31 transplants is caused more by a deficiency in the protein component than by a deficiency in the flavin prosthetic group.

Transplants of Walker carcinosarcoma 256 showed no activity of any of the 3 enzymes studied. This is somewhat difficult to interpret since we have no normal control tissue for this tumor with which to compare the results.

SUMMARY

1. An investigation of *d*-amino acid oxidase apoenzyme, uricase, and choline oxidase has been made in two transplanted rat tumors.

2. The transplants of hepatoma 31 and the nuclei isolated from them showed only 3 per cent of the *d*-amino acid oxidase apoenzyme activity of the normal liver tissue. Addition of the coenzyme of *d*-amino acid oxidase caused this activity to increase to 12 per cent of the normal value.

3. Transplants of hepatoma 31 contained only 5 per cent of the uricase activity of normal liver tissue. The nuclei isolated from these transplants contained about the same amount of uricase as the whole tumor tissue.

4. Choline oxidase could not be detected in either the transplants of hepatoma 31 or in nuclei isolated from them.

5. *d*-Amino acid oxidase, uricase, and choline oxidase could not be detected in Walker carcinosarcoma 256 transplants.

I wish to express my appreciation to Professor W. R. Bloor and Dr. A. L. Dounce for their advice and encouragement throughout the course of this work.

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Specific Injurious Action of Alloxan Upon Pancreatic Islet Cells and Convoluted Tubules of the Kidney. Comparative Study in the Rabbit, Dog, and Man

Attempted Chemotherapy of Insulin-Producing Islet Cell Carcinoma in Man*

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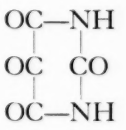
Agents capable of causing injury and necrosis of specific categories of cells are of interest in connection with the possibilities of chemotherapy of cancer. The recent report by Dunn, Sheehan, and McLetchie (1) that alloxan, the ureide of mesoxalic acid and a component of the uric acid molecule, the structural formula of which is,



causes selective necrosis of the islets of Langerhans in the pancreas and of the epithelium of the convoluted tubules in the kidneys in rabbits is of unusual importance.

These authors stated that all of "over a dozen" animals died within 4 or 5 days of injection or were killed before final collapse developed. There was an initial hyperglycemia followed by hypoglycemia, within several hours. Histologic study revealed coagulation necrosis of the islet cells and necrosis of the epithelium of the convoluted tubules; other tissues were normal.

In confirmation of the above studies the following experiments in 8 rabbits were performed by the authors. A summary of the protocols follows.[1]

Rabbits NI and NII received 30 and 60 mgm. of alloxan per kilo intravenously on each of 10 successive days. No deleterious effects were noted and there was no elevation in blood glucose or N.P.N. levels. The animals were killed 4 days after the last injection and histologic study of the kidneys and pancreas showed no abnormalities.

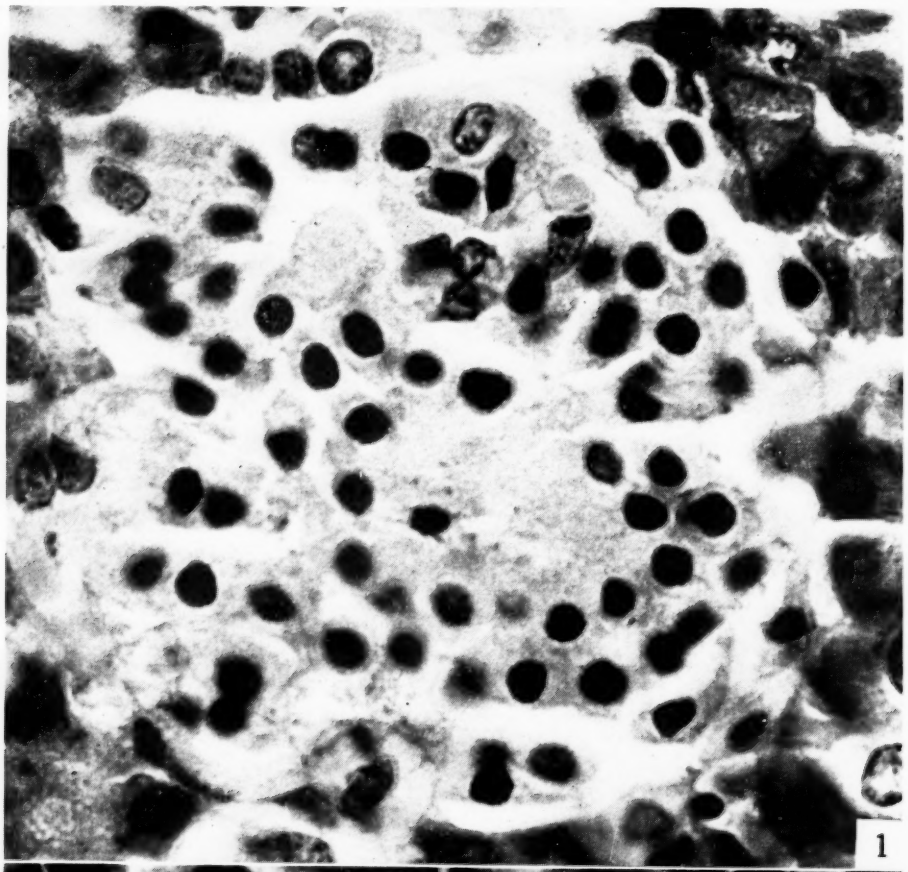
Rabbit Br. received 175 mgm. of alloxan per kilo intravenously and died during the fourth night following injection. Postmortem changes precluded histologic study.

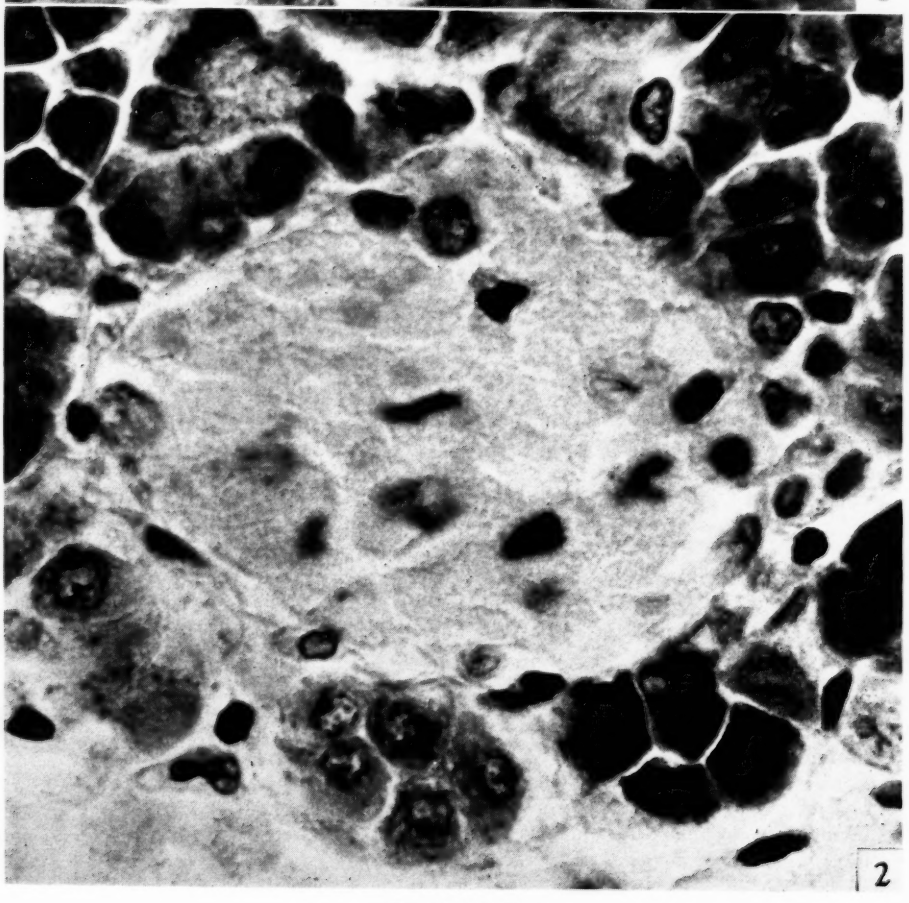
Rabbit B received 200 mgm. of alloxan per kilo intravenously and was found dead 17 hours later, with legs outstretched and head drawn sharply backward. Histologic study (5 per cent formalin fixation, hematoxylin and eosin stain) of the pancreas revealed varying degrees of coagulation necrosis in the islets (Figs. 1, 2, and 3). In some instances a few cells were affected, most of the others showing pyknotic nuclei and shrunken cytoplasm. In other instances almost all the islet had undergone coagulation necrosis, many of the cell shadows being still discernable. Scattered shrunken cells with pyknotic nuclei were present in almost every islet that was not necrotic. The acinar tissue exhibited no abnormal changes and a very striking feature was the absolute lack of leukocytic infiltration in or about the necrotic islets. The kidneys had undergone no abnormal changes. Sections of liver and adrenal revealed no abnormalities. All other viscera were macroscopically normal.

Rabbit BII received 360 mgm. of alloxan per kilo intravenously and died 4 days later. Just before death the blood glucose was 485 mgm. per cent, and the N.P.N., 196 mgm. per cent. Upon histologic study of the pancreas a striking feature was the first impression that islets were very rare; more careful examination under higher powers of the microscope revealed scattered contracted and degenerated islets that appeared as small collections of shrunken, closely applied cells with pyknotic nuclei. Again there was no evidence of leukocytic infiltration in or about the degenerated and necrotic islets. The other cells in the pancreas—acinar, duct, and interstitial tissue—appeared

* This investigation was conducted under a grant from the O. C. Miller Fund for Cancer Research.

[1] Alloxan, a white crystalline powder, was dissolved in 15 to 20 cc. normal saline just before injection. Stock solutions were not prepared. One sample of the powder was apparently not fresh, for the prepared solution was deep pink in color; this was not employed. The solutions should exhibit only a very light pink tinge.





FIGS. 1 AND 2

normal. There was extensive coagulation necrosis of the convoluted tubules of the kidneys.

Rabbits Bl. R. and A. each received 500 mgm. of alloxan per kilo. Both died suddenly in approximately 1½ hours after injection. Histologic study of the pancreas in each instance revealed in some islets a few scattered shrunken cells with pyknotic nuclei. Many of the islets showed no abnormal changes, nor did the kidneys.

Rabbit Gr. received 100 mgm. alloxan per kilo and survived, remaining constantly in good general condition. Hyperglycemia was present on the third day (212 mgm. per cent), a slight hypoglycemia (74 mgm. per cent) developed on the fourth day, hyperglycemia again appeared on the seventh day, and thereafter the animal presented normal blood sugar levels. The blood N.P.N. remained within normal limits at all times. The daily blood sugar and N.P.N. levels are presented in Table I. The animal was killed on the 20th day after injection for histologic study. No abnormalities were present in the pancreas and kidneys or in other organs.

TABLE I: EFFECT OF 300 MGM. ALLOXAN INJECTED INTRAVENOUSLY IN RABBIT GR., WT. 3 KILOS, ON BLOOD GLUCOSE AND N.P.N. LEVELS. INJECTION CARRIED OUT ON FIRST DAY AFTER INITIAL BLOOD SAMPLE WAS WITHDRAWN

	Days												
	1	2	3	4	5	6	7	8	9	10	11	12	20
Blood glucose mgm., per cent	97.5	95.0	212.0	74.0	149.0	171.0	136.0	143.0	—	123.0	—	105.0	125.0
Blood N.P.N. mgm., per cent	43.8	30.5	—	24.4	29.6	22.1	36.6	39.8	—	36.2	—	27.5	40.3

* Normal blood glucose for rabbit 100-140 mgm. per cent.

The effects of intravenous injections of alloxan were then studied in dogs, as such observations in these animals have not been previously reported. For purposes of discussion the animals may be divided into 3 groups: I. Those in which death occurred a few hours after injection. II. Those in which there was survival for a few days. III. Those in which there was prolonged survival.

Group I. 5 dogs.—Death in a few hours following injection of a single dose of alloxan. One dog (Sp.) received 100 mgm. per kilo and died 1 hour and 40 minutes later. Two others (BII and P.W.) received 200 mgm. per kilo and died 45 minutes later. Shortly after injection they became seriously depressed and did not stand. Blood glucose levels (fasting) before injection were 62 and 78 mgm. per cent respectively and at death 171 and 474 mgm. per cent respectively; the N.P.N.'s were within the normal range. The fourth dog (L. Br.) received 300 mgm. per kilo and died 3 hours later. The blood glucose before injection was 73 mgm. per cent and at death was 47 mgm. per cent. The fifth dog (Bl. T.) received 300 mgm. of alloxan per kilo and was found dead 17 hours later. All these animals exhibited a copious sanguineous frothy discharge in the nostrils and mouth. At necropsy large patches of hemorrhagic consolidation were present in the lungs.

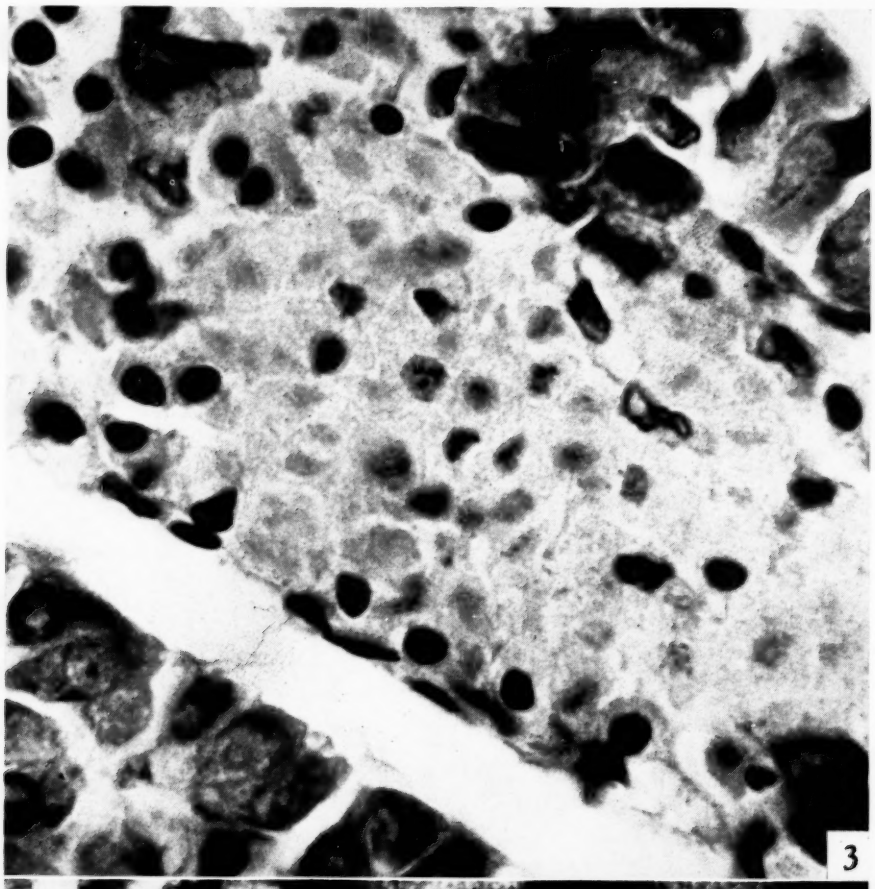
Histologic study of the pancreas in the 2 animals dying 45 minutes after injection did not reveal significant changes in the islets, although at death the blood sugar as stated above was 171 and 475 mgm. per cent respectively. In the animal that died 1 hour and 45 minutes after injection many islets appeared normal, but in a number of instances some of the cells were shrunken, and the cytoplasm was densely eosinophilic. In other islets several of the cells in the centers seemed shrunken together and separated from the normal appearing cells about them. In the animal that died 17 hours after injection, postmortem changes were advanced but discrete islets were recognized in the pancreas. In all instances the kidneys were normal. Sections of the lungs taken from the areas of consolidation showed the alveoli filled with an eosinophilic granular precipitate with occasional erythrocytes.

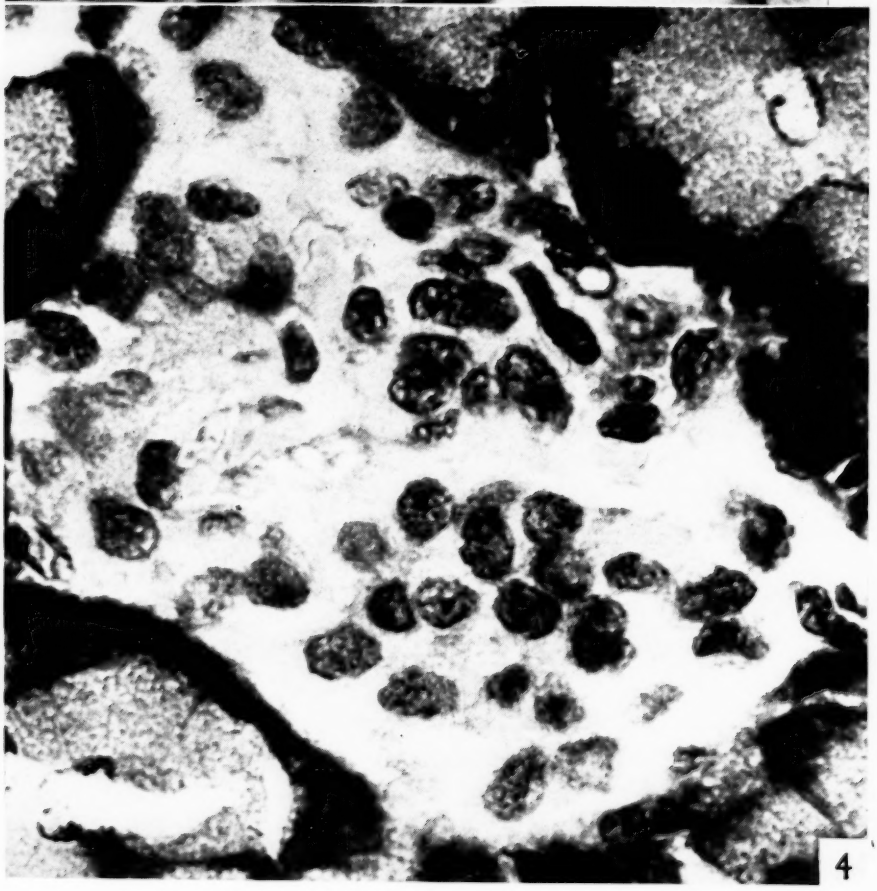
Group II. 6 dogs (554, Bl., Tan., Bri., Col., Mid.).—Death on the fourth to sixth day following 1 to 5 daily injections of alloxan totalling 100 to 500 mgm. per

DESCRIPTION OF FIGURES 1 AND 2

FIG. 1.—Essentially normal islet of pancreas from control rabbit for comparison with Figs. 2 and 3. Mag. × 1180.

FIG. 2.—Islet of Langerhans in pancreas of rabbit B that received 200 mgm. of alloxan per kilo intravenously and died 17 hours later. Most of the islet cells show coagulation necrosis, "shadows" of individual cells are discernible, some pyknotic nuclei still present. The cells of the surrounding acinar tissue are unaffected. Note absence of leukocytic infiltration. Mag. × 1180.





FIGS. 3 AND 4

kilo. The protocols are summarized in Table II. In each animal blood glucose levels were abnormally high 48 hours after injection of alloxan; the maximum levels only are given in the table.

Histologic study (fixation in 5 per cent formalin, hematoxylin and eosin stain) of the pancreas in each instance gave the impression upon preliminary inspection with the lower powers of the microscope that islets were unusually rare. This was due to the fact that in the normal pancreas (Fig. 4) the islets are composed of relatively clear cuboidal or polyhedral cells that contrast sharply with the darker staining acini, whereas in these instances the islet cells seemed shrunken and much more closely applied than normally (Fig. 5). Not infrequently the nuclei appeared agglomerated and the individual cell boundaries were not discernible. Occasionally an individual cell looked necrotic, with dense eosinophilic cytoplasm and pyknotic nucleus, or the cytoplasm exhibited abnormal vacuolization. Extensive coagulation necrosis as seen in the rabbit's islets was not observed in these specimens.

The majority of the convoluted tubules in the kidneys were necrotic (Fig. 6) and were converted into eosinophilic casts that filled the entire space of the tubule; viable lining cells were not present in these segments. In a single exceptional case the kidneys appeared normal (dog Mid.). This animal had received a total dose of 120 mgm. per kilo in 2 days, and by the fourth day the blood glucose was 267 mgm. per cent. On the sixth day it was 192 mgm. per cent, the blood N.P.N. remaining normal. He was found dead on the morning of the seventh day after the first injection and sections of the kidneys revealed no abnormal changes. This dog, 7 months old, had been born and reared in the animal room and appeared to be in excellent condition throughout the period of study subsequent to injection. Sections of lung, liver, and adrenal were also normal.

Group III. 4 dogs.—Prolonged survival. Dog 549 received three injections of 50 mgm. of alloxan per kilo on 3 consecutive days. The daily blood glucose levels are shown in Fig. 7. The initial rise was not great, reaching 148 mgm. per cent on the third day with subsequent fall to normal levels, then a secondary rise developed on the seventh and eighth days to 162 and 151 mgm. per cent respectively, following which the glucose level again fell to normal. On the 19th, 20th, and 21st days the injections were repeated with another brief rise in blood sugar to a maximum of 229 mgm. per cent and subsequent fall to normal levels. Throughout the 31 day observation period blood N.P.N. remained normal, and the animal was in good condition.

TABLE II: MAXIMAL FASTING BLOOD GLUCOSE AND N.P.N. IN DOGS GROUP II FOLLOWING INTRAVENOUS INJECTION OF ALLOXAN

Animal	Weight, kilos	Total dose, mgm./kilo	Maximum blood glucose	Maximum blood N.P.N.	Survival
554	10.3	250 divided into 5 daily injections	263 (5th day)	115.0 (5th day)	Killed 6th day, seriously depressed
Bl.	8.15	100 one injection	477 (before death)	300.0 (before death)	Died 5th day
Tan.	7.85	100 " "	270 " "	96.8 " "	Died 4th day
Bri.	5.10	100 " "	461 (5th day)	245.0 (5th day)	Died 5th day
Col.	10.00	150 divided into 2 daily injections	267 (6th day)	286.0 (6th day)	Died 6th day
Mid.	10.6	120 " " " " "	267 (5th day)	50.5 (5th day)	Found dead 7th day

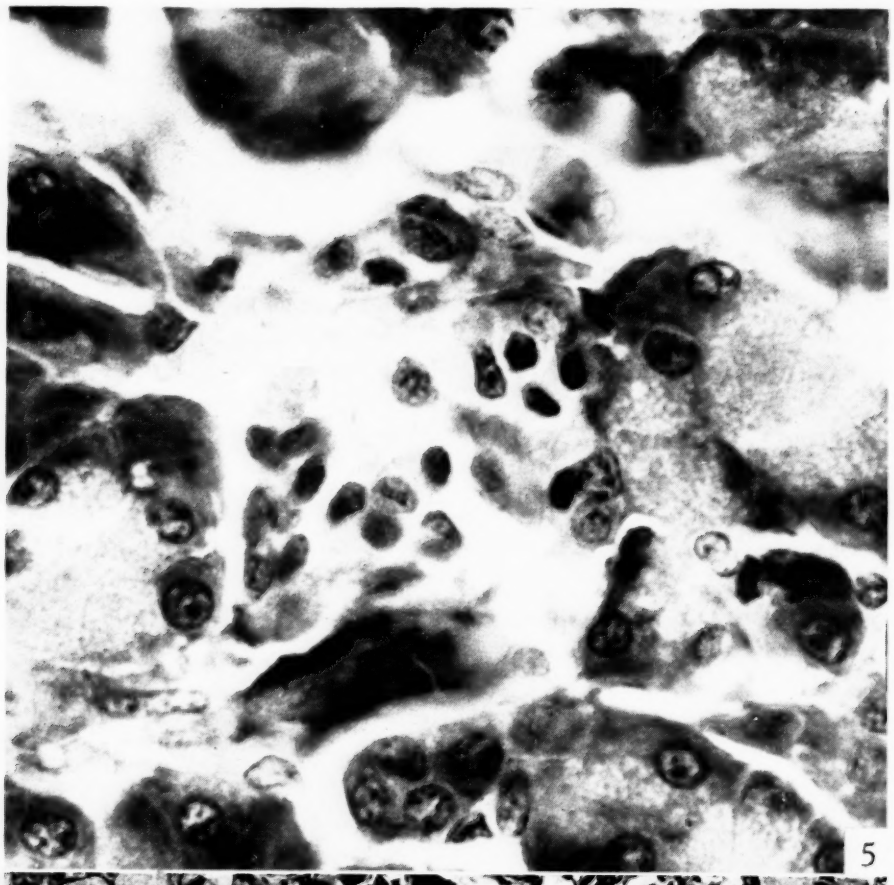
Dog Sh. received injections of 75 mgm. per kilo on 2 consecutive days (Fig. 8). On the third day the blood sugar rose to 285 mgm. per cent, fell to 101 mgm. in 2 days, rose to 192 mgm. on the ninth day and then returned to normal after the 13th day. A second single injection of 75 mgm. per kilo intraperitoneally was given on the 21st day followed by a rise to 184 mgm. per cent 48 hours later with subsequent fall to almost normal levels. Elevated blood N.P.N. levels were observed at irregular intervals. A severe purulent infection developed in the right forelimb on the 25th day, from which the animal did not recover and died 4 days later. The final blood glucose and N.P.N. were determined on a sample drawn a few minutes after death.

Dog Bu. received 50 mgm. per kilo on 2 consecutive days and exhibited after the second day an elevated blood glucose, 281 mgm. per cent. As seen in Fig. 9 there were variations in the blood glucose levels during

DESCRIPTION OF FIGURES 3 AND 4

FIG. 3.—Another islet in pancreas of rabbit B (Fig. 2). Necrosis of islet cells is not as advanced as in the islet shown in Fig. 2 The nuclei of a number of the cells are still discernible but "faded." Mag. × 1180.

FIG. 4.—Normal appearing islet of Langerhans from pancreas of a dog for comparison with Fig. 5. Mag. × 1180.



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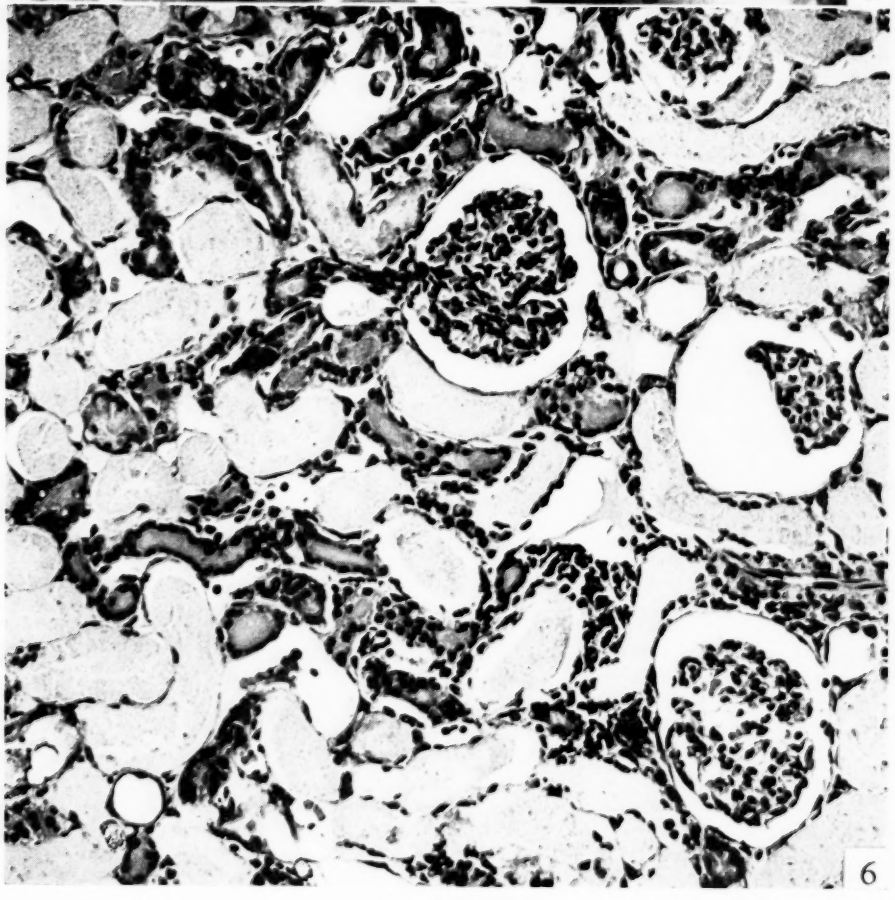

6

FIGS. 5 AND 6

the first 10 days but this remained for the most part above normal. After the 14th day there was a sustained high blood glucose level ranging between 200 and 250 mgm. per cent.

The blood N.P.N. during this period was consistently within the normal range.

Dog 553 received 25 mgm. per kilo of alloxan and exhibited no abnormal variations in the blood glucose or N.P.N. levels. It was killed on the tenth day after injection and histologic study of the pancreas and kidneys showed no abnormalities. Obviously the dose was too small to be effective.

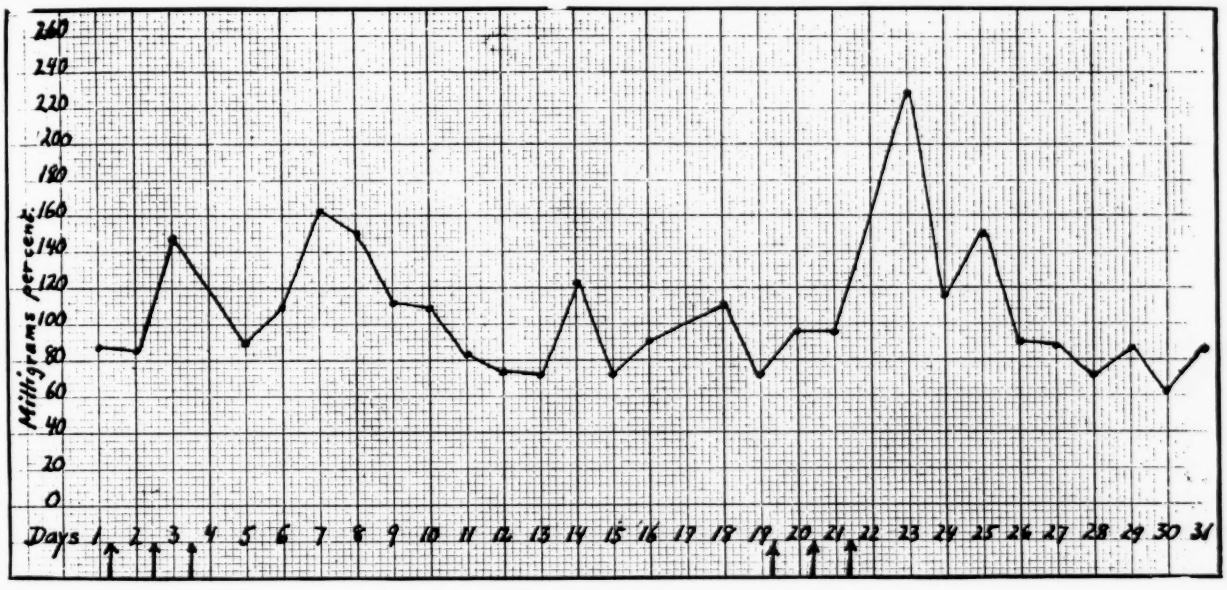

FIG. 7.—Dog 549, wt. 5.6 kilos. Three daily injections of 280 mgm. of alloxan (arrows). Total dose 150 mgm. per kilo. Moderate irregular hyperglycemia during first 10 days. Repetition of injections (arrows) on 19th, 20th, and 21st day. Irregular hyperglycemia 22nd to 25th day, with subsequent return to normal. N.P.N. levels within normal range throughout period of observation.

Because of the selective deleterious action upon the islet cells, it was decided to administer alloxan to a patient from whom a large insulin-producing islet cell tumor was removed in January, 1940, as previously reported in the literature (2). Two and one-half years after operation he presented recurring attacks of hyperinsulinism with hypoglycemic shock. In July, 1942 an exploratory laparotomy revealed multiple superficial and deep hepatic metastases and peritoneal spread of the previously excised large pancreatic tumor. During the fall and winter of 1942-43 attacks became increasingly frequent and finally occurred daily. X-ray therapy to the abdomen was ineffective. Attacks were avoided by ingestion of food at 3 hourly intervals. At the end of 3 hours without food, but before an attack developed, the blood sugar was usually 14 mgm. per cent. The blood N.P.N was normal. This patient, weighing 112 kilos, received a total of 220 mgm. of alloxan per kilo in divided doses over a period of 5 days; doses of 2.8 gm. to 11.2 gm. were dissolved in 500 cc. normal saline and injected intravenously. Each injection was given over a period of 45 minutes to 1 hour.

He had been having 2 to 5 attacks of hyperinsulinism a day. Following these injections he returned home and had only 6 attacks, one on each of 6 days, and was 8 days without attacks. This aroused considerable interest; he returned to the hospital at intervals between August 1 and November 1, 1943, and received injections over 3 to 4 day periods totalling as high as 670 mgm. to 1 gm. per kilo. After each series there would be freedom from attacks for 10 to 21

DESCRIPTION OF FIGURES 5 AND 6

FIG. 5.—Islet of pancreas from dog Bri., who received 100 mgm. alloxan per kilo intravenously and died 5 days later. The blood glucose rose to 461 mgm. per cent and blood N.P.N. rose to 245 mgm. per cent. Note shrunken appearance of islet, and shrunken appearance of some of individual cells with pyknotic nuclei. Mag. × 1180.

FIG. 6.—Kidney from dog Tan., who received 100 mgm. alloxan per kilo intravenously and died four days later. Blood glucose rose to 270 mgm. per cent, and blood N.P.N. prior to death was 96.8 mgm. per cent. Note granular casts in spaces previously occupied by convoluted tubules; illustrating specific necrosis of convoluted tubule epithelium resulting from injection of alloxan. Mag. × 200.

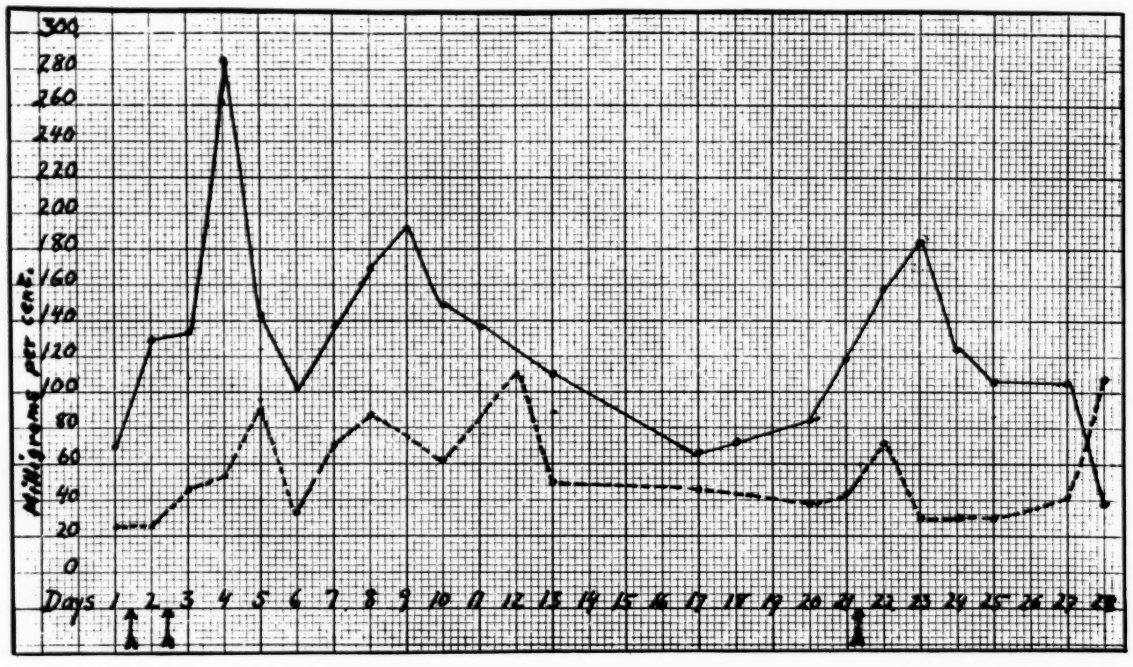

FIG. 8.—Dog Sh., wt. 7.7 kilos, 575 mgm. alloxan intravenously on first and second days (arrows). Total dose approximately 150 mgm. per kilo. Daily variations in fasting blood glucose level (solid line) and in blood N.P.N. level (broken line). Variable hyperglycemia during first 13 days, and blood N.P.N. levels abnormally high during most of this period. After blood glucose and N.P.N. levels had returned to normal a second injection of 575 mgm. of alloxan 21st day, resulting again in hyperglycemia and elevated N.P.N. Animal died on 28th day of infection of forelimb. Last blood glucose and N.P.N. levels determined on blood withdrawn about 15 minutes after death from vena cava (no clots).

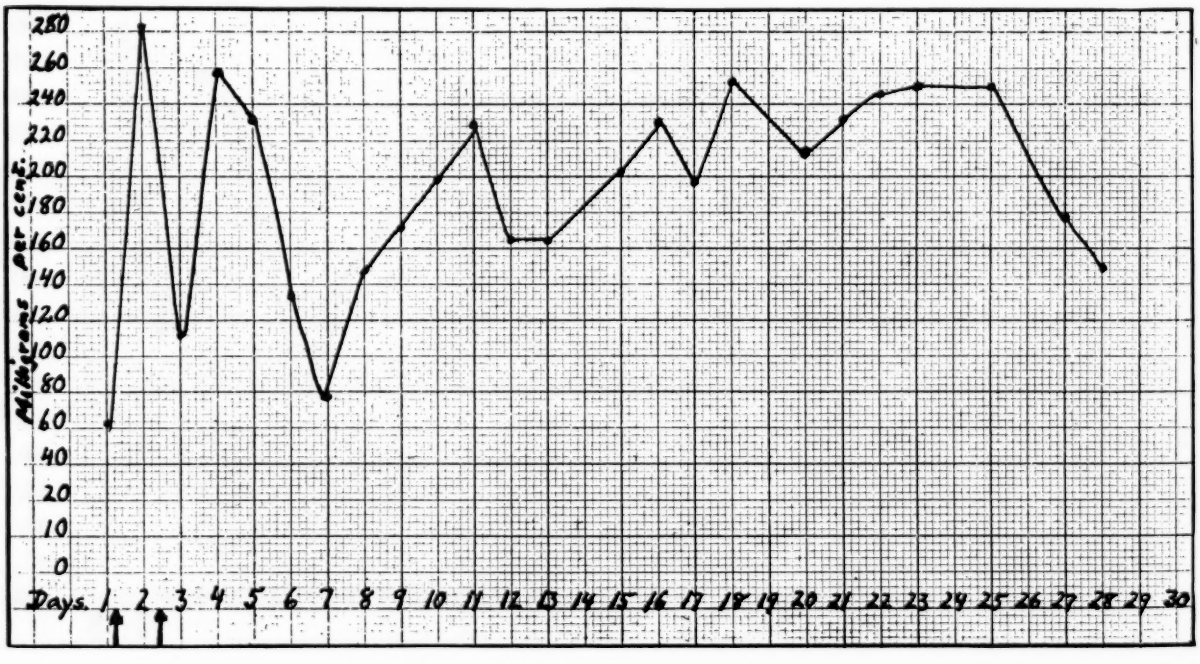

FIG. 9.—Dog Bu., wt. 9.8 kilos. Received 500 mgm. alloxan intravenously on first and second days (arrows). Total dose approximately 100 mgm. per kilo. Daily fasting blood glucose level. Diabetic state more or less continuous after injection due to injury of pancreatic islet cells. Hyperglycemia maintained for longer period than shown. On 31st day blood glucose level was 239 mgm. per cent. Blood N.P.N. continuously within normal range.

days. Blood sugar levels as high as 298 mgm. per cent were noted. Blood N.P.N. remained within normal limits. These studies will be reported in greater detail in a subsequent paper. (In press, Journal of the American Medical Association.)

Another patient with carcinomatosis, primary in the stomach, received 2 daily injections of 200 mgm. of alloxan per kilo, totalling 400 mgm. per kilo, without evident disturbances. The blood sugar and N.P.N. levels remained within the normal range.

A third patient with carcinomatosis, primary in the pancreas, of duct cell origin, received a total dose of 950 mgm. of alloxan intravenously during a 6 day period. The individual daily doses were: first to third days inclusive, 50 mgm. per kilo; fourth day, 300 mgm. per kilo; sixth day, 500 mgm. per kilo. The injections resulted in no objective or subjective disturbances and the blood sugar and N.P.N. levels remained within normal limits during and subsequent to the injection period.

A fourth patient with carcinomatosis received 600 mgm. per kilo, and without preliminary hyperglycemia, developed in 5 hours hypoglycemia, 16 mgm. per cent, and died after apparent recovery from the attack. Necropsy showed early degenerative changes in the islets, and degenerative changes in the hepatic cells.

DISCUSSION

In the rabbit and dog the pancreatic islet cells and epithelium of the convoluted tubules of the kidney are specifically injured by alloxan injected intravenously. Physiologically this is manifested by hyperglycemia and uremia. Investigations of this "chemical" diabetes are being carried out by Goldner (3) and Gomori (Department of Medicine, University of Chicago). Their studies were begun simultaneously with, but independently of, the investigations reported in this paper.

The pancreatic islets of the rabbit would appear more sensitive to alloxan than those of the dog, in that extensive coagulation necrosis of islet cells occurs in the former but was not observed regularly to a similar degree in the latter receiving comparable doses, although histologic evidence of islet cell damage was present.

Doses of the order of 200 to 500 mgm. per kilo are usually fatal in both rabbit and dog; doses of 100 to 150 mgm. per kilo are not always fatal and the hyperglycemia in the animals that survived was transitory in 3 of 4 instances (2 dogs, 1 rabbit). In the fourth animal (dog) it was prolonged, continuing for at least 28 days.

The pancreatic islets are more sensitive to alloxan than is the epithelium of the convoluted tubules of the kidney since in no instance was there an elevation of blood N.P.N. without hyperglycemia, whereas in one surviving rabbit (Gr.) two brief periods of hyperglycemia developed without elevation of blood N.P.N. In one dog (549) similar findings were obtained and in a dog (Bu.) with continuous hyperglycemia for 28 days, blood N.P.N. was consistently normal. Furthermore, in another dog (Mid.), dying on the seventh day after injection of alloxan, the blood glucose had risen to 267 mgm. per cent, while the blood N.P.N. remained within normal limits. Histologic study of the kidneys showed no abnormalities.

Jacobs (4) and Dunn, Sheehan, and McLetchie (1) noted that during a period of 5 to 9 hours immediately following the injections there was first hyperglycemia, followed by hypoglycemia. Evidence of a hypoglycemic state shortly after injection of alloxan was also noted by the authors in some of the dogs described above. In dog L. Br. the blood glucose was 73 mgm. per cent at the time of injection and at death, 3 hours later, was 42 mgm. per cent. In another dog (Bri.) the blood glucose was 67 mgm. per cent at the time of injection, 107 mgm. per cent 2 hours later, 148 mgm. per cent 4 hours later, 150 mgm. per cent 6 hours later, and 19.5 mgm. per cent the next morning, when the animal was seriously depressed. The following day it was 418 mgm. per cent, above which level it remained for 3 days until the animal died. In dogs Tan., and Bl. the blood glucose was 32 and 40 mgm. per cent respectively 18 hours after injection of 100 mgm. of alloxan per kilo.

The mechanism of the wide variation in blood glucose levels and eventual diabetogenic action of alloxan is not clear. It is certainly more complex than simple arrest of insulin production by the islets. Where total pancreatectomy is performed in a dog, and no insulin administered, maximal blood sugar levels do not develop for 48 hours or more and then are usually not greater than 200 to 300 mgm. per cent. Not only were blood sugar levels of 312, 461, 474, and 477 mgm. per cent observed in some of the dogs but the hyperglycemia developed in relatively brief periods following injection. The most striking example was in dog P. W., whose blood sugar was 78 mgm. per cent at injection (200 mgm. per kilo) and 45 minutes later at death was 474 mgm. per cent. This would suggest a possible combination of neutralization of insulin throughout the organism with outpouring of glucose into the blood from the tissues. The brief period of hypoglycemia observed following the initial and almost immediate hyperglycemia might represent, as suggested by Dunn and his associates, an overcom-

pensating hypersecretion of insulin by islet cells during the initial stages of injury.

The basis for the relatively greater resistance of man to the action of alloxan is not apparent. A variety of explanations immediately suggest themselves. Among them is the fact that alloxan is a component of the uric acid molecule and thus the difference in the final phases of purine metabolism between man on the one hand, and rabbits and dogs on the other, might afford the key to the solution of the problem. In man uric acid is the end product of nucleoprotein catabolism. In the rabbit and dog uric acid is further converted to allantoin by means of uricase. Possibly in the latter system alloxan is a factor in the production of a substance that specifically injures islet cells and the epithelium of the convoluted tubules. In any event, such speculations are permissible in anticipation of further investigation of this question.

SUMMARY

1. Alloxan, the ureide of mesoxalic acid, when injected intravenously, produced specific necrosis of islet cells in the pancreas and epithelium of the convoluted tubules of the kidneys in rabbits. These observations are in confirmation of the work of Dunn, Sheehan, and McLetchie.

2. In dogs, intravenous injection of alloxan also injured specifically the islet cells and convoluted tubules of the kidney. The islet cells in these animals, however, did not exhibit the extensive coagulation necrosis observed in the rabbits.

3. In the dog, injury to islet cells and tubular epithelium in the kidney was manifested physiologically by diabetes mellitus and uremia. Following injection there was a brief period of hyperglycemia followed by a brief period of hypoglycemia; at the end of 48 hours hyperglycemia again was present and persisted for varying intervals.

4. The islet cells appeared more sensitive to the effects of alloxan in that some animals exhibited hyperglycemia and relatively transient or no uremia.

5. In dogs, 200 to 500 mgm. of alloxan per kilo was fatal in from 1 hour to 6 days, the animals having died with definitely elevated blood glucose and blood N.P.N. After total doses of 100 to 150 mgm. per kilo the animals sometimes survived with transitory diabetes and with or without transitory impaired renal function. In one dog a sustained diabetes mellitus (over 28 days) without elevated blood N.P.N. was observed.

6. Four human patients with carcinomatosis, one presenting an insulin-producing islet cell carcinoma of the pancreas, received intravenous injections of alloxan. Transitory beneficial effects were observed in the patient with insulin-producing islet cell carcinoma, following injection of 600 mgm. to 1 gm. per kilo, in that attacks of hyperinsulinism were abolished for 10 to 20 days following each series of injections, whereas before the injections he had 2 to 5 severe attacks a day. In the other three patients also, comparably larger doses of alloxan were given than in the dogs and rabbits, with effects on the blood in sugar in only one instance. Hence it appears that the human subject is much more resistant to the action of alloxan than the dog or rabbit.

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Metaplasia of the Bronchial Epithelium in Rats Following Application of Benzpyrene*

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INTRODUCTION

Despite the recent advances in the treatment of bronchogenic carcinoma this disease presents the clinician with many unsolved problems. Primary carcinoma of the lung constitutes 5 to 10 per cent of all malignant tumors (2, 13). With improvement in methods of diagnosis at least 75 per cent of cases are recognized during life, whereas 30 years ago probably only 10 per cent were diagnosed clinically. Since the performance of the first total pneumonectomy by Graham (10) for the removal of a lung tumor, a satisfactory surgical approach to the problem has been developed. Yet the end result in a large series of cases is very discouraging for the reason that 70 to 90 per cent of all patients with bronchogenic carcinoma are inoperable when the diagnosis is made (12, 7, 4, 8). Vigorous attempts are being made to diagnose this lesion, as well as all types of cancers, in the early stage so that treatment may be instituted in time to cure the patient.

ETIOLOGY

Very little is known about the cause of bronchogenic carcinoma. Many etiological factors have been postulated, but actually very little has been proved. Excessive epithelial regeneration of the bronchial mucosa may occur following influenza, but in some countries, notably Iceland, where influenza has been common, bronchogenic carcinoma is a rarity. The inhalation of irritating substances is often suggested as a cause. Well known is the Schneeberg lung cancer, which is probably a lymphosarcoma. A high incidence of these lung tumors is found among the workers in the cobalt mines of Saxony. Relatively intimate contact with the mines is essential, as the families of the workers who live nearby are not prone to develop lung tumors. Recently miners of pitchblende (radium) at Joachimsthal in Bohemia have been reported to show an abnormally high incidence of lung tumors. Mechanical irritation seems not to be a factor as patients with silicosis and pneumoconiosis develop tuberculosis rather than tumors. Chemical irritation or the action of radioactive agents have not been disproved as possible causes of bronchogenic carcinoma.

In recent years several investigators have attempted to produce bronchial tumors in experimental animals. A variety of animals and methods of administration of carcinogenic agents have been employed with indifferent results. Campbell (5) exposed mice to an atmosphere of road dust containing 2 per cent tar, and 74 per cent of his animals developed lung tumors as compared with 14 per cent of the control group. Seelig and Benignus (16) used coal smoke in a similar experiment on white mice. Eight per cent of their animals developed carcinoma of the lung in contrast to 2 per cent in the control group. Several investigators have reported a higher incidence of lung tumors in mice whose skin had been painted with tar, than in control animals (11, 15, 3, 14). Andervont (1) produced lung tumors in mice by the subcutaneous injection of 1,2,5,6-dibenzanthracene. Shimkin (18) confirmed Andervont's work, using the carcinogen intravenously and also produced lung tumors in mice by the intratracheal injection of methylcholanthrene and dibenzanthracene (17). Intratracheal administration of carcinogens into larger animals has been less successful.

Since most primary lung tumors in man arise from bronchial epithelium we felt that the carcinogenic agent should be applied directly to the tracheobronchial tree. Fareed (9) attempted to spray benzpyrene into rabbits through the bronchoscope, but the animals did not tolerate the frequent anesthetics necessary for repeated bronchoscopies. He then attempted to establish permanent tracheotomies in rabbits, but most of the animals died of infection or suffocation. Carlson (6) developed a technic for the production of bronchial fistulae in rats and rabbits. We used his technic with a few modifications to produce bronchial fistulae, which were then subjected to the repeated application of a carcinogenic substance.

METHOD

Young adult male and female white rats were used throughout the experiment. No spontaneous lung tumors have been observed to occur among the animals

* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

of this colony although we have used them for over 2 years. An occasional breast tumor has been noticed.

A bronchial fistula was prepared by a two stage operation. The first stage consisted of exteriorizing the left lung in a subcutaneous pocket. Under mask ether anesthesia the left pleural space was opened in the fifth interspace. The lung was grasped with a tissue forceps and brought out between the ribs, which were then closed about the hilum of the lung by two paracostal sutures. The pleural space was aspirated with a syringe and needle, and the skin closed over the exteriorized lung. This operation can be done in 3 to 5 minutes, and practically no deaths result if a few simple precautions are taken. The paracostal sutures may be placed before the pleural space is opened. This allows more rapid closure, which is an important factor when positive pressure anesthesia is not used. As soon as the pleural space is closed the pneumothorax should be aspirated. The lung in a rat is very friable and considerable care must be used in tying the paracostal sutures as they may occlude the blood supply to the lung. On the other hand they must not be so loose that the hilum of the lung will retract into the pleural space at the subsequent stage. Extreme caution must be exercised in pulling the lung out of the pleural space or a tear in the lung with fatal hemorrhage may result.

After 4 to 10 days the second stage may be performed. Open mask ether anesthesia is again employed. The old incision is reopened and the lung freed. Adhesions that may have formed are readily broken down. A rubber band tourniquet is placed about the hilum of the lung, and 70 to 80 per cent of the lung resected The bronchus is cannulated with ordinary wrapping cord following which the vessels are ligated with fine silk. The torniquet is then removed and no closure is made. The mortality from this operation is rather high; uncontrollable bleeding may result, and the open wound is prone to become infected. Deaths may occur as late as 4 weeks postoperatively from infection. If the animal survives, a good bronchial fistula usually results.

The rats have to be observed daily as the fistula tends to close rapidly once the string falls out. The string is changed at 3 day intervals or whenever it falls out. Within 3 to 14 days after the second stage, treatment of the fistula is begun. The carcinogen used in this study was 3,4-benzpyrene in olive oil. A piece of wrapping cord was saturated in the carcinogen and inserted into the fistula. It was changed at 3 day intervals, or sooner if it fell out. The animals were then sacrificed at intervals to determine what changes took place.

Carlson (6) has published drawings to show the stages of the operation, as well as bronchograms made by injecting iodized oil into the fistula.

Protocol No. 1
Rat No. 2 Male
July 14, 1941

First stage exteriorization of the left lung.—The animal was placed in an ether jar until completely anesthetized. He was then removed and the anesthesia continued with open drop ether. The left thorax was shaved and painted with 3½ per cent iodine. The animal was pinned to an operating board and draped with sterile towels.

A 3 cm. incision was made over the left fifth interspace and the muscles severed down to the ribs. Two paracostal sutures of fine silk were placed around the fifth and sixth ribs. The pleural space was opened. The left lung was grasped with a tissue forceps and gently teased onto the chest wall. The inferior pulmonary ligament tore easily and there was no bleeding. The paracostal sutures were tied so as to draw the fifth and sixth ribs about the hilum of the lung. Approximately 6 cc. of air was aspirated from the pleural space. The skin was then closed over the exteriorized lung with interrupted fine silk sutures.

July 15. Listless, but able to move about.

July 16. Active. No evidence of inflammation about the wound.

July 18. Some peeling of the skin due to the iodine, but no infection.

July 22. Second stage exteriorization of the left lung.

Anesthesia was conducted as during the first stage. The skin was painted with iodine and the animal placed on the operating board. The sutures were removed and the skin incision reopened. The lung looked perfectly normal except for two small areas of atelectasis. Practically no adhesions were present. A rubber band was applied snugly about the hilum, and about 70 per cent of the lung resected. A piece of wrapping cord was inserted into the bronchus following which bleeders were ligated with fine silk. On removing the rubber band no bleeding was noted. The animal was placed in his cage.

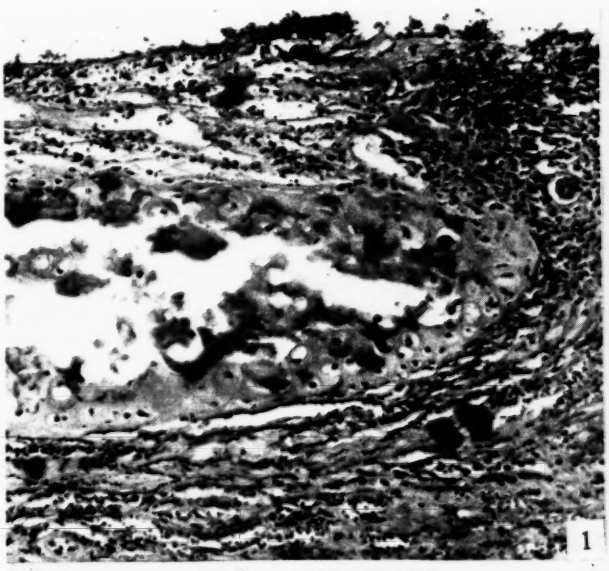
July 27. The string was removed and replaced.

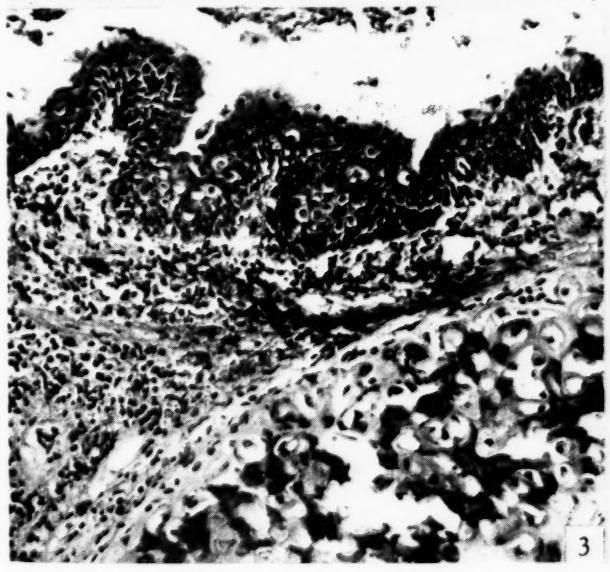
July 29. The fistula seemed adequate so treatment with a string saturated in benzpyrene was begun. These treatments were continued until October 2, 1941, at 1 to 3 day intervals.

At this time it was noted that the animal was not eating well and was losing weight. He was therefore sacrificed.

At autopsy a bronchial fistula leading to the trachea was demonstrated. No tumors were seen in the fistula and there was no infection in the chest. The abdominal contents were normal. A 1 cm. brain abscess was found which evidently accounted for the loss of weight.

Microscopic sections of the bronchial fistula demonstrated definite squamous metaplasia of the bronchial epithelium with pearl formation (Fig. 4).





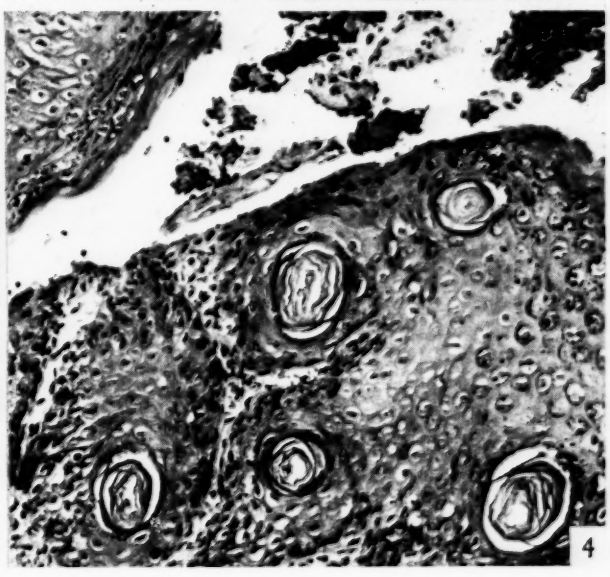


FIG. 1.—Rat No. 8. Treatments were carried out for 33 days. Epithelium completely destroyed; infiltration of round cells in submucosa.

FIG. 2.—Rat No. 31. Animal died 7 days after treatment was begun. Epithelium piled up about 5 to 6 cells deep. Heavy inflammatory reaction in submucosa.

FIG. 3.—Rat No. 20. Animal survived 26 days. Epithelium 7 to 10 cells deep and beginning to take on characteristics of squamous epithelium. Note bronchial cartilage in lower part of field.

FIG. 4.—Rat No. 2. Sacrificed after 67 days. The stratified squamous epithelium represents the most advanced change obtained. Pseudo-pearl formation present.

RESULTS

Some 150 rats were used in the course of the experiment. Some died at one of the two operations, and many of infection before treatment with the carcinogenic agent could be started. After the trend of the change in the bronchial epithelium had been established many animals that died from 1 to 14 days after application of the carcinogen was begun were examined at autopsy, but in an effort to avoid unnecessary waste of material, no sections were made. We were able, however, to demonstrate a relatively rapid change in the character of the bronchial epithelium following the application of benzpyrene. Animals autopsied as early as 5 to 7 days after the first application showed a change in the bronchial epithelium to a type not unlike the transitional lining seen in the genitourinary system. Cells were 4 to 5 deep, cuboidal

in shape, with a deeply staining, rounded nucleus and relatively scanty cytoplasm. If applications were continued for 3 weeks an early type of stratified squamous epithelium was produced. The cells were 5 to 10 deep, but formed no keratin or pearls. Animals that died or were sacrificed about 8 weeks after applications were begun developed typical stratified squamous epithelium in the bronchus, and in some there was modified pearl formation. We were unable to carry the change any further despite the fact that in some animals applications were continued for over 100 days. In no case was a tumor seen on gross examination, and no invasion of the underlying tissues was ever demonstrated microscopically. In a relatively high percentage of the group the epithelium was destroyed, presumably by the intense inflammatory reaction.

TABLE I

Rat number	Length of treatment, days	Type of epithelium found on section
1	75	Destroyed
2	67	Stratified squamous, with pearls
3	22	Stratified squamous, 5 to 10 cells deep. No keratin
4	46	Destroyed
6	49	"
8	33	"
9	20	"
10	118	None
Then no treatment	205	
13	6	Transitional, 5 to 8 cells deep
15	9	None
16	35	"
18	13	"
19	39	"
20	26	Early stratified squamous
21	108	None
No treatment	205	
22	11	Transitional, 10 cells deep
23	49	Stratified squamous
30	5	Transitional
31	7	"

DISCUSSION

The experimental method described above may be open to some criticism. First, only about 50 per cent of the animals developed a good fistula, and a fistula will tend to close spontaneously unless watched constantly. Second, the influence of infection is difficult to control, and all the wounds were infected in some degree. However, we have followed animals that had the exteriorization procedures without application of a carcinogen for considerable periods of time and these animals have never shown the metaplastic changes described above. Finally, there is always the possibility that skin will grow up to the margin of the open bronchus and epithelialize the bronchus. We feel fairly certain that this was not the case in this experiment for two reasons: (a) the change in the bronchial epithelium was gradual; and (b) an attempt was made to preserve a cuff of lung about the fistula so that it did not come directly in contact with the skin.

The white rats used in this experiment are known to be resistant to the more common carcinogenic agents, and spontaneous tumors of any type are relatively uncommon in the strain. However, we were interested to learn if a fairly potent carcinogenic agent, such as benzpyrene, would cause any epithelial overgrowth. A very definite metaplastic change, first to pseudotransitional epithelium, and finally to a fairly well differentiated stratified squamous epithelium occurred in 8 weeks. We were unable to demonstrate further changes even though experiments were continued for as long as 200 days. Two modifications in the technic might be attempted. It should be possible in rats to apply carcinogenic agents repeatedly by injection directly into the trachea. When metaplasia occurs the change might be further augmented by the use of filterable viruses obtained from the common wart.

SUMMARY AND CONCLUSIONS

Definite metaplasia of bronchial epithelium was produced by the use of 3,4-benzpyrene applied to a bronchial fistula in white rats. Within 1 week a pseudotransitional epithelium had developed, which changed to a stratified squamous type by the end of 8 weeks. In some cases modified pearl formation was produced. Although benzpyrene was applied 3 times a week for as long as 100 days no gross or microscopic tumors were observed during the experiment.

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Abstracts

Experimental Research, Animal Tumors

Polycyclic Aromatic Compounds. Cook, J. W. [The Royal Cancer Hospital (Free), London, England] *Annual Reports on the Progress of Chemistry for 1942 Issued by the Chemical Society,* **39**:155-191. 1943.

A review, the various sections of which deal with the hydrocarbons of coal tar, carcinogenic hydrocarbons, structure, molecular compounds, stereochemistry and steric factors, synthesis, and reactions.—E. L. K.

Morphology and Growth of Subcutaneous Tumors Induced with Carcinogenic Hydocarbons in Strain C3H Male Mice. Shimkin, M. B., and Bryan, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:25-35. 1943.

Of 415 subcutaneous tumors induced by 8.0 to 0.00195 mgm. of methylcholanthrene, dibenzanthracene, and benzpyrene in male mice of the C3H strain, 411 were spindle cell sarcoma, 2 were carcinoma, and 2 consisted of mixed carcinoma and sarcoma. One-third of the sarcomas had prominent muscle cell elements. The larger doses of methylcholanthrene and benzpyrene induced a relatively early appearance of the 4 carcinomas and also yielded sarcomas which grew more rapidly on the average than did tumors induced with lighter injection. Prominent muscle cell elements appeared more frequently in sarcomas elicited with the higher doses of the carcinogen.

Histologic variations did not exert a significant influence upon the latent time and growth rate of the tumors.—K. R. P.

Aerobic Oxidation of Aromatic Hydrocarbons in the Presence of Ascorbic Acid. The Reaction with Anthracene and 3:4-Benzpyrene. Warren, F. L. [The Chester Beatty Research Inst., The Royal Cancer Hosp. (Free), London, England] *Biochem. J.,* **37**:338-341. 1943.

Hydrocarbons (naphthalene, phenanthrene, anthracene, 3,4-benzpyrene, cholanthrene and methylcholanthrene) undergo changes when in solution (80% acetone in water) together with ascorbic acid in the presence of oxygen. The products formed by this reaction from anthracene and 3,4-benzpyrene were found to be respectively 9,10-anthraquinone, and a mixture of 3,4-benzpyrene-5,8-quinone and -5,10-quinone together with an alkali-soluble compound, probably phenolic, which could not be isolated. The reaction does not take place in the absence of oxygen; it is inhibited completely by KCN (M/100) or HPO_3 (5%) and is not affected by H_2O_2, nor as a rule by cupric ions, though the last named sometimes produce a slight acceleration. Dihydroxy maleic acid acts in the same way as ascorbic acid, hence the reaction is probably due to the enediol grouping. It seems probable that the oxidizing agent is dehydroascorbic acid or a peróxide derived from it (cf. the inhibition of the autoxidation of benzaldehyde or heptaldehyde by 3,4-benzpyrene with production from the latter of one or more quinones, Wasley and Rusch, *Cancer Research,* **2**:422-424. 1942).—E. L. K.

An Experimental Study of Chromatography. Weil-Malherbe, W. [North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-upon-Tyne, England] *J. Chem. Soc. London,* 303-312. 1943.

This investigation began during the development of a method for the micro-estimation of 3,4-benzpyrene and other hydrocarbons in extracts containing the nonsaponifiable matter of animal tissues. In the interest of quantitative recovery the procedure had to be devised in such a way that it was capable of coping with all the concentrations of hydrocarbons likely to occur, down to the limits of accurate measurement; on the other hand, any undue extension of this margin had to be avoided to insure the greatest possible purification.

Simple chromatographic systems consisting of one adsorbent, one adsorptive, and one solvent were studied by plotting the quantity of adsorptive in the filtrate against the total volume of the filtrate. The resulting "elution curve" is of sigmoid form. A large number of data upon the adsorption of 3,4-benzpyrene are given, and a method for fluorimetric estimation of the compound is described.—E. L. K.

Mucocele of the Appendix and Pseudomyxoma Peritonei. A Clinical and Experimental Study. Grodinsky, M., and Rubnitz, A. S. [Univ. of Nebraska, Coll. of Med., Omaha, Nebr.] *Nebraska M. J.,* **27**:201-205. 1942.

The health of a patient was permanently improved after removal of a mucocele of the appendix. In experiments with rabbits this lesion was readily produced by ligation of the appendix after irrigation of the lumen with saline. If the cleansing process was not carried out, gangrene followed ligation. Intraperitoneal injection of the contents or of the wall of the lesion into other rabbits resulted in implantation and the growth of the deposits. One rabbit developed masses that regressed spontaneously.—E. E. S.

Attempt to Induce Formation of Fibroids with Estrogen, in the Castrated Female Rhesus Monkey. Vargas, L., Jr. [Carnegie Inst. of Washington, Baltimore, Md.] *Bull. Johns Hopkins Hosp.,* **73**:23-30. 1943.

Subcutaneous implantations of estradiol failed to cause fibroid tumors or disseminated miliary nodules in 4 oöphorectomized monkeys.—J. G. K.

Depolymerases for Yeast and for Thymus Nucleic Acids in Normal and Neoplastic Tissues. Greenstein,

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Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

J. P. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:55-61. 1943.

The activity of ribonucleodepolymerase (RD) and of desoxyribonucleodepolymerase (DRD) in water extracts of various rat, mouse, and rabbit tissues was determined. The method is described, and evidence is presented to show that the action of the enzymes is limited to depolymerization of the nucleates.

The 2 enzymes varied together in the different tissues. The activity of both enzymes was lower in 2 types of mouse lymphoma than in normal mouse lymph nodes; about the same in transplanted rat hepatoma and in normal and regenerating rat liver; sometimes higher in mouse hepatoma than in normal mouse liver; identical in fetal and adult rabbit liver; identical in the spleens of normal rats and in the enlarged spleens of rats bearing hepatoma 31.—H. Q. W.

Note on the Colloid Osmotic Pressure of the Serums of Rats Bearing the Transplanted Jensen Sarcoma. Greenstein, J. P., and Thompson, J. W. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:63-64. 1943.

The authors show that there is a progressive decrease in the colloid osmotic pressure and in the percentage of protein in the serums of rats bearing Jensen sarcoma.—H. Q. W.

Differential Inhibition between Normal and Tumor (Crown Gall) Tissue in Beet Roots. Michaelis, M., Levi, I., and Hibbert, H. [McGill Univ. Montreal, Canada] *Science*, **98**:89-90. 1943.

The oxygen uptake of beet root tumors induced by inoculation with *Phytomonas tumefaciens* is inhibited 20 to 23% by 0.0166M resorcinol. In the case of normal root tissues from healthy noninfected beets the inhibition is 12 to 14%. By cyanide (0.0166M) the inhibition is 79 to 80% for tumor, and 84 to 86% for normal tissue. Cyanide and resorcinol together inhibit the oxygen uptake of tumor 86% and that of normal tissue 92%.—R. B.

On Specific Chemotherapy for Cancer. Eggers, H. E. [Univ. of Nebraska, Coll. of Med., Omaha, Nebr.] *Nebraska M. J.*, **27**:133-136. 1942.

This is a further report on the effects upon the growth of malignant tumors of toxic derivatives of glucose administered with insulin. The results with transplanted rat sarcoma R2426 were less striking than those previously reported (*Arch. Path.* **18**:507. 1934). Injection of tetra-methyl- and tetra-ethyl-arsonium gluconate and the gluconate of an arsenical base, produced by heating under pressure methyl iodide and arsenic trioxide, had no permanent effect on tumor growth in rats. Injections of the arsenical gluconate and insulin given to a patient with carcinoma of the prepuce and inguinal metastases were followed by regression of the lymph node metastases. The primary carcinoma was excised, and there was no sign of recurrence 6 years later. The second patient was free of tumor 3 years after similar injections together with amputation of the distal half of the penis because of squamous cell carcinoma. The third patient died of coronary disease 1 month after cauterization of a squamous cell carcinoma of the penis, biopsy of an inguinal lymph node containing tumor, and injections. At autopsy the remaining nodes showed much disintegration of the tumor cells. In the fourth patient, injections were given after irradiation of a primary carcinoma of the dorsum of the hand and excision of an axillary metastasis. There was no recurrence during the subsequent 4 years. In addition, many patients showed no perceptible effect even after repeated doses, although a few had temporary regression of metastases. The author concludes that there is an indication of a specific effect on cancer tissue of injections of toxic derivatives of glucose, together with insulin.—E. E. S.

The Failure of H 11 to Inhibit Growth of Tumors in Mice. Gye, W. E., Ludford, R. J., and Barlow, H. [Imperial Cancer Research Fund, Mill Hill, London] *Brit. M. J.*, **2**:65-67. 1943.

The preparation known as H 11 is an extract of urine which is recommended for the treatment of cancer, and was introduced by J. H. Thompson and his fellow workers, who, in tests upon mice bearing tumors, give 1 cc. intraperitoneally twice daily. The authors found no inhibitory action upon (1) Strong A carcinoma growing in Strong A mice; (2) methylcholanthrene C57 sarcoma growing in C57 mice; (3) mammary carcinoma 63, and spindle-celled sarcoma 37, growing in mixed stock mice; nor (4) upon mammary carcinoma 63 in RIII mice. The results of tests on tissue cultures on carcinomas 63 and Strong A, and C57 sarcoma were also negative.—E. L. K.

Observations on the Use of H 11 in Carcinoma. Kidd, H. A. [Kingston County Hosp., Kingston-on-Thames, England] *Brit. M. J.*, **2**:67-69. 1943.

The author gave a total of over 16,000 injections of H 11 in 51 advanced cases of cancer; the greatest number of injections given to a single patient was 1,116. "In most instances the condition was inoperable, and usually the patient was in poor general health, although there was considered to be a sufficiently reasonable expectation of life to justify treatment. This period of time was assessed at six weeks in accordance with the statement made by Thompson that objective signs of improvement do not usually occur until such an interval of treatment has elapsed. Some cases had other treatment, including operation and radium, but with few exceptions they were cases in which the operation or radium therapy was considered to be insufficient to eradicate the local disease.

There is some evidence that in a few cases the rate of growth has been slowed up or inhibited, but in no case confirmed by section has, as yet, any growth disappeared as the result of H 11 therapy only."—E. L. K.

H 11 for Cancer. Correspondence. *Brit. M. J.*, **2**:149-150. 1943.

Correspondence arising from the 2 papers abstracted above.

Thompson, J. H., Holt, P. F., and Jones, R. F. The authors criticise the results of Gye and his associates in that the Twort carcinoma was not used and the treatment was not sufficiently prolonged.

Gye, W. E., and Ludford, R. J. To this Gye and Ludford reply "that malignant tumours of mice which we have treated with H 11 continued to grow and killed the mice at the same rate as our untreated controls."

Scott, J. A. The author considers that 11 of Kidd's 51 cases suggest "that the exhibition of H 11 might have exercised some influence."—E. L. K.

H 11 for Cancer. CORRESPONDENCE. *Brit. M. J.*, **2**:211-212. 1943.

CURTIS, F. A report of "an apparently very successful clinical result of treatment" in a case of cancer of the bladder with extensive metastases.

LOWE, E. C. A letter in support of the method.

STEEL, W. A. A series of cases (number not stated), inoperable or resistant to radium therapy, was treated; "in many the treatment was prolonged to the limit of the patients tolerance. . . . . In none of the cases was life prolonged nor did the growth seem to be in any way inhibited. . . . . Every one of the patients submitted to this treatment at this hospital died, and I came to the conclusion that, however effective the substance was in the control of the transplant growth in mice, it had no effect clinically." —E. L. K.

The Effect of Injections of H 11 on the Growth of Mouse Tumours. WOODHOUSE, D. L. [Cancer Research Laboratory, Med. Sch., Edgbaston, Birmingham, England] *Brit. M. J.*, **2**:231-232. 1943.

In 54 mice bearing grafted dibenzanthracene sarcomas, carcinoma 63, or induced benzpyrene skin epitheliomas no inhibitory effect on the tumor growth was found after prolonged injections of the urine preparation H 11 or silimar extracts.

Two sarcomas removed from mice after such injections were successfully grafted into other animals, and histological examinations of treated tumors showed no difference from control material.—Author's summary.

H 11 for Cancer. HANNAN, J. H. CORRESPONDENCE. *Brit. M. J.*, **2**:314. 1943.

The writer has treated inoperable cases (number not stated) and has "formed the conclusion that the extract was harmless and seemed to exercise some inhibitory action in certain cases." An especially favorable result in a case of carcinoma of the vulva is described.—E. L. K.

H 11 for Cancer. RADNOR, I. CORRESPONDENCE. *Brit. M. J.*, **2**:403. 1943.

H 11 was used in "fairly advanced cases of (1) rectal cancer; (2) sigmoid cancer; (3) secondaries following ovarian cancer; (4) secondaries following breast cancer; (5) ulcerating breast cancer. Treatment in each case failed and the patient died from cancer. In the first 4 cases treatment was prolonged and intensive. In case 5 treatment with H 11 was discontinued after 2 months as no improvement was apparent."

H 11 ointment, recommended for the treatment of warts, was used in one case for 3 months without effect.—E. L. K.

Some Investigations upon the Nature of the Resistance of an Inbred Line of Fowls to the Development of the Rous No. 1 Sarcoma. CARR, J. G. [Inst. of Animal Genetics, Edinburgh, Scotland] *Brit. J. Exper. Path.*, **24**:127-132. 1943.

By selection from a pedigreed Brown Leghorn flock it was found possible to produce a line of birds, known as the "non-susceptible" or N-S line, which was highly resistant to the agent of the Rous No. 1 sarcoma. Inoculation of the agent into 6 week old fowls of the N-S line typically produced small tumors, most of which regressed between the 14th and 22nd days. This resistance was present in day old chicks, although to a lesser extent, and it was found not to be due to serum antibodies. (It is recalled that while antibodies to the Rous No. 1 agent are often present in "normal" fowls, they are usually found only in older animals; and Amies obtained no indication of their presence in young chicks.) The resistance was exhibited also to implanted cells of the Rous sarcoma, and to the cells of a fowl sarcoma (GRCH/15) that had been induced by chemical means.—A. H.

Serologic and Anaphylactic Reactions of the Cathepsins of Normal and Neoplastic Tissues. MAYER, M. E., and BARRETT, M. K. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:65-73. 1943.

The results of precipitin, complement-fixation, and anaphylactic tests indicated that the cathepsins of normal rat liver and transplanted hepatoma 31 are different proteins with some common protein groups. These tests showed also that the cathepsins of Jensen sarcoma and transplanted hepatoma 31 are more closely related in chemical structure than are the cathepsins of hepatoma 31 and normal liver. Rat kidney and spleen cathepsin reacted with low titers to the antiserum to the cathepsins of normal liver, hepatoma 31, and Jensen sarcoma. Anaphylactic tests with guinea pigs confirmed the results of the precipitin and complement-fixation tests. Much more normal liver cathepsin than hepatoma cathepsin was needed to produce shock in an animal sensitized with hepatoma cathepsin.—K. R. P.

An Adaptation of the Transparent-Chamber Technique to the Mouse. ALGIRE, G. H. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:1-11. 1943.

A technic is described for making prolonged (30-40 day) microscopic *in vivo* observations on tumor growth in the mouse. The procedure involves the use of a transparent chamber that is inserted into a skin flap on the animal's back. Microimplants of tumor tissue made at the time the chamber is inserted or later can be watched under magnifications as high as 500 diameters. Complete immobilization of the microscopic field is obtained by the use of a specially designed mouse holder.—K. R. P.

Microscopic Studies of the Early Growth of a Transplantable Melanoma of the Mouse, Using the Transparent-Chamber Technique. ALGIRE, G. H. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:13-20. 1943.

The author reports preliminary results obtained with the transparent-chamber technic in a study of the growth of the Cloudman melanoma (S 91). Of 10 attempted implants, 5 were successful, and the proliferation of one of these was followed as long as 36 days. During the first 20 days the tumor tissue showed little growth. It was surrounded successively by leukocytes, macrophages, and multinucleated cells, the latter being derived from macrophages and not from tumor cells. Vascularization began on the 20th day and proceded rapidly. Simultaneously with this process the migration and proliferation of tumor cells became accelerated. The vascular bed of the melanoma was stabilized 8 days after its inception.—K. R. P.

Long-Term Cultivation *in Vitro* of a Dibenzanthracene Mouse Sarcoma. JACOBY, F. [Dept. of Physiology & Cancer Labs., Med. School, Birmingham, England] *Nature, London*, **152**:299. 1943.

A pure strain of sarcoma cells derived from a dibenzanthracene mouse tumor was grown *in vitro* for slightly

longer than 2 years (38 passages). The morphology of the cultures showed little variation during this period. Explants were grown in Carrel flasks on a coagulum of hen plasma and chick embryo juice, and were fed twice weekly with a hen serum-chick embryo juice-Tyrode mixture. There was considerable variation in the extent and rate of growth. In certain sera there was found to occur a substance toxic to the sarcoma cells but not to homologous macrophages, which endangered the survival of the former. Cultures were inoculated into mice from time to time, but the frequency of takes decreased with prolonged cultivation *in vitro*. After 393 days growth *in vitro* the injection of 5 cultures per mouse into 3 mice failed to produce a tumor. Two cultures left at the end of the period of cultivation also were inoculated with negative results.—R. J. L.

Characteristics of a Liposarcoma Grown in Vitro. MURRAY, M. R., and STOUT, A. P. [Coll. of Physicians and Surgeons, and Presbyterian Hosp., New York, N. Y.] *Am. J. Path.*, **19**:751-763. 1943.

Cultures were made from the axillary metastasis of a liposarcoma, by means of the Maximow double-coverslip, lying-drop method. The cultures grew profusely and regularly after a lag period of 18 to 24 hours. Fat, bright, spindle-shaped, discrete cells with a round or oval vesicular nucleus and one or two prominent nucleoli made up the characteristic growth. Binuclearity was common, and in the later stages of cultivation some exceedingly bizarre, lobated, and fragmented nuclei made their appearance. These were more prominent in irradiated cultures. Six figures show the growth and its cells in culture.—J. G. K.

The Production of Multipolar Mitoses in Normal Embryonic Chick Cells. STILWELL, E. F. [Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *Science*, **98**:264-265. 1943.

Numerous multipolar mitoses, chiefly triasters, were produced in cultures of 8 day normal embryonic chick heart muscle, incubated at 37½ to 42° C. for 7 to 8 days. The usual hanging drop technic was used, and a total of 98 cultures was made. In addition to triasters, other aberrant mitotic forms observed were: "resting" cells with two or more nuclei, and chromosome vesicles (as many as 16 in one cell). The cells exhibiting these abnormalities were unusually large, and the cultures in general showed vigorous growth.

It is pointed out that the possibility of production of multipolar mitoses in normal somatic cells *in vitro* provides an approach for the study of the relationship between aberrant mitoses and malignancy.—J. B. B.

Neural Induction in Epidermal Explants in Liquid Medium. SHEN, S. C. [Zoological Lab., Cambridge, England] *J. Exper. Biol.*, **19**:5-10. 1942.

A small disc of presumptive epidermis was dissected from each of a number of axolotl gastrulae in the region exactly opposite the point of gastrulation. Each fragment was cultivated separately. Twenty-five explants were cultivated in Holtfreter's solution alone, and an equal number in the same saline with the addition of the water-soluble carcinogen, Na-1,2,5,6-dibenzanthracene-α-β-endosuccinate in a concentration of 10 μgm./cc. All explants were cultivated for at least 10 days before being fixed and prepared for histological examination. Of the 25 explants cultivated in saline alone, 16 survived, and all of these consisted exclusively of clusters of epidermal cells lacking any specific orientation. Of the 25 explants cultivated in the presence of the carcinogen 18 survived, and of these 11 (approximately 60%) exhibited definite and extensive neurulation. The induced neural structures were in the form of "more or less radially symmetrical neural balls," in most cases hollow in the center, with no regional differentiation.

In a "Note added in Proof" C. H. Waddington refers to a paper by L. G. Barth (*J. Exper. Zoöl.*, **87**:371. 1941.), which appeared after Shen's paper was in press. Barth has shown that ectoderm from the amphibian gastrula cultivated in salt solution may develop into neural tissue, in the total absence of any tissues from the organization center, when special precautions are taken to preserve the polarity of the isolated tissue, and when the explants are large. His results, however, confirm the conclusion of Holtfreter and others that small explants such as those cultivated by Shen in the normal way, without any attempt to preserve polarity, do not form neural tissue.—R. J. L.

A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. V. Absence of Sex Influence When a Large Dose of a Carcinogen Is Administered. STRONG, L. C. [Yale Univ., Sch. of Med., New Haven, Conn.] *Arch. Path.*, **36**:58-63. 1943.

In about 1,000 normal mice, the subcutaneous injection of 1 mgm. of methylcholanthrene dissolved in sesame oil induced tumors in approximately the same percentage of males and females. A brief survey of the pertinent literature indicates that estrogens and similar hormones do not influence the induction of tumors by carcinogenic chemicals.—J. G. K.

Study of a Spontaneous Mouse Rhabdomyosarcoma. NETTLESHIP, A. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **3**:563-568. 1943.

A spontaneous, transplantable, mature type rhabdomyosarcoma of strain C mice is described for the first time. Histologically, it is similar to such tumors in man, described previously, and in domesticated animals, but contains more adult type of cells with intact cross striations and myofibrillae. Many cells appear to be in contraction. No nerve endings can be proved to be present.—Author's summary.

Structure and Histogenesis of Tumors of the Aortic Bodies in Dogs. With a Consideration of the Morphology of the Aortic and Carotid Bodies. BLOOM, F. [Flushing, N. Y.] *Arch. Path.*, **36**:1-12. 1943.

Two cases of tumors occurring spontaneously in the region of the base of the heart in dogs were encountered in a series of 500 necropsies. The growths had the same anatomic location and histologic characteristics as the aortic bodies. They are described in detail and illustrated with 10 figures.—J. G. K.

Clinical and Pathological Reports

Diagnosis—General

Maintenance of the Sedimentation Rate as a Test for Malignant Disease. Apter, L., Hull, E., and Adams, C. C. [Louisiana State Univ. Sch. of Med., and Charity Hosp. of Louisiana, New Orleans, La.] *Am. J. M. Sc.*, **206**:168-174. 1943.

Maintenance of the initial sedimentation rate in blood stored for 24 hours proved unreliable as a criterion for the presence of malignant disease.—J. G. K.

Radiation—Diagnosis and Therapy

Roentgen Diagnosis of Malignant Nasopharyngeal Tumors. Belanger, W. G., and Dyke, C. G. [New York Neurological Inst., New York, N. Y.] *Am. J. Roentgenol.*, **50**: 9-18. 1943.

In 14 cases of proved malignancy, tumors of the nasopharynx were studied radiographically, and osseous and soft tissue changes described. The importance of a stereoscopic basal examination is emphasized.—E. H. Q.

Dosage System for Roentgen Therapy. Camiel, M. R., and Blatz, I. H. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Am. J. Roentgenol.*, **50**:67-75. 1943.

Simple methods are presented for calculating the tumor dose when roentgen factors are known, and for determining the amount of radiation to be given to each field when the tumor dose is prescribed.—E. H. Q.

The Results of Roentgen Therapy for Metastatic Neoplasms. Deucher, W. G. [Zurich, Switzerland] *Am. J. Roentgenol.*, **50**:197-203. 1943.

Statistical data are given on the fate of 432 patients with distant metastases from malignant tumors. Some of the patients were treated with roentgen irradiation, others remained untreated. Local palliation was brought about in 70% of treated patients; this corresponds to 45% of the total number of cases. The duration of life was generally longer for the treated patients, and only in this group did survival periods of more than 3 years occur. The most important criterion of the usefulness of the treatment is the restoration and prolongation of the patient's well-being. This aim was reached in about one-half of all cases treated. —E. H. Q.

Carcinoma of the Head of the Pancreas Demonstrating the New Signs in Roentgen Diagnosis. Case Report. Friedman, P. S. [Jefferson Hosp. and Med. Coll., Philadelphia, Pa.] *Am. J. Roentgenol.*, **49**:197-198. 1943.

Roentgenograms are presented of a carcinoma of the head of the pancreas, with a recently described type of mucosal change in the duodenum.—E. H. Q.

Radiation Treatment of Lymphangioma. Holmes, G. W., and Hawes, L. E. [Massachusetts General Hosp., Boston, Mass.] *Am. J. Roentgenol.*, **49**:799-802. 1943.

From a review of 12 cases the authors conclude that there is only one form of lymphangioma, the verrucous type of lymphangioma simplex, that responds favorably to irradiation given in doses that do not cause permanent injury to normal tissue. All other forms either do not respond at all or require a dose larger than can be given with safety. Infection is a definite hazard in the treatment of these cases, particularly of the large tumors of the neck. For this reason the taking of biopsies and the insertion into the tumor of radium in the form of seeds or needles should be discouraged.—E. H. Q.

A System of Tumor Dosage, Records and Technique as Employed at the Brooklyn Cancer Institute. Howes, W. E., and Bernstein, L. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Am. J. Roentgenol.*, **50**:76-88. 1943.

Standard technics are presented for treatment of a wide variety of tumors. Methods for computing and recording the tumor dose, and tables of tumor doses are included.—E. H. Q.

Roentgen Rays in the Treatment of Malignant Tumors of the Kidney in Adults. Kerr, H. D., and Stephens, R. L. [State Univ. of Iowa, Coll. of Med., Iowa City, Iowa] *Am. J. Roentgenol.*, **50**:204-206. 1943.

Thirty-seven adults were treated for neoplasm of the kidney either by x-rays and surgery or by radiation alone. Twelve patients are living 5 years or more without evidence of disease; 8 of these had preoperative irradiation. Only 1 patient who was treated by radiation alone survived for this period; this method is, therefore, not recommended.—E. H. Q.

The Elimination of Irradiation Injuries in the Treatment of Cancer of the Cervix. Martin, C. L. [Dallas, Texas.] *Am. J. Roentgenol.*, **49**:494-503. 1943.

A radiation technic for the treatment of carcinoma of the cervix is presented with detailed data on 149 patients treated by this method during a 5 year period. The author states that the end results are equal to those reported by other clinics, and that serious irradiation sequelæ are fewer. —E. H. Q.

Seminoma of the Testis from the Standpoint of Roentgen Treatment. Nash, L. A., and Leddy, E. T. [Mayo Clinic, Rochester, Minn.] *Am. J. Roentgenol.*, **50**:162-196. 1943.

An analysis of 103 proved cases of seminoma fails to provide clinical criteria for accurate diagnosis of the disease; the only certain diagnostic procedure is biopsy. Seminoma is the commonest type of testicular neoplasm; the authors consider it a highly undifferentiated adenocarcinoma. Hormonal analysis is of no definite help in either diagnosis or treatment. Roentgen therapy is the method of choice. —E. H. Q.

An Instrument for Inserting Multiple Capsules of Radium within the Uterus in the Treatment of Corpus Cancer. Nolan, J. F., and Arneson, A. N. [Washington Univ. Sch. of Med., and Barnard Free Skin and Cancer Hosp., St. Louis, Missouri] *Am. J. Roentgenol.*, **49**: 504-518. 1943.

In an attempt to improve the method of radium treatment of cancer of the uterine fundus, a simple instrument was devised for the insertion of multiple capsules. The technic is described.

Sixty-nine cases treated with radium and subsequent hysterectomy were studied with regard to number and position of radium sources, local radiation effect observed in

the operative specimen, and clinical result. The time elapsed is too brief to permit final evaluation of the treatment. Preoperative x-ray irradiation together with radium applied within the uterus by a multiple capsule technic, are recommended.—E. H. Q.

Radiation Therapy in Cancer of the Esophagus. An Analysis of Eighty-Five Cases Observed During the Last Decade. POHLE, E. A., and BENSON, R. R. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Am. J. Roentgenol.*, **50**:89-100. 1943.

A review of 85 cases of cancer of the esophagus, treated by radiation at the University of Wisconsin shows that the average duration of life of patients after admission was 6.7 months; about 10% lived longer than this. Although most patients are admitted in an advanced stage of the disease, even then roentgen therapy offers palliation.—E. H. Q.

SKIN AND SUBCUTANEOUS TISSUES

Pigmented Papilloma of Skin. FOX, R. A. [St. Louis Univ. Sch. of Med., St. Louis, Mo.] *Arch. Path.*, **36**:195-200. 1943.

Five cases are presented in detail with 7 figures. The growth is benign, pigmented, and of epithelial origin; it is unrelated to the tumors composed of nevus cells.—J. G. K.

Tumors of Sweat Glands. GATES, O., WARREN, S., and WARVI, W. N. [Harvard Cancer Commission and New England Deaconess Hosp., Boston, Mass., and Pondville State Hosp. for Cancer, Wrentham, Mass.] *Am. J. Path.*, **19**:591-631. 1943.

Extensive review of the literature and discussion, with 9 figures.—J. G. K.

Lipoma-Like Basal Cell Epithelioma. SUTTON, R. L., JR. [Univ. of Kansas Med. Sch., Kansas City, Mo.] *Arch. Dermat. & Syph.*, **48**:176-178. 1943.

Case report, with 5 figures.—J. G. K.

Epithelial Cysts and Cystic Tumors of the Skin. WARVI, W. N., and GATES, O. [Harvard Cancer Commission and Massachusetts State Tumor Diagnostic Service, Boston, Mass.] *Am. J. Path.*, **19**:765-783. 1943.

Review based on 566 epithelial cysts examined during 20 years, with numerous references to the literature, and 3 figures.—J. G. K.

NERVOUS SYSTEM

Astrocytoma of the Left Cerebral Hemisphere with a Psychoneurotic Reaction. COHEN, L. A. [Veterans Administration, North Little Rock, Ark.] *M. Bull. Vet. Admin.*, **20**:94-96. 1943.

Case report with autopsy findings.—M. E. H.

Intracranial Calcification. Report of an Exceptionally Large Calcified Tumor. LIGHT, R. A. [Vanderbilt Univ. Sch. of Med., Nashville, Tenn.] *Ann. Surg.*, **117**: 309-312. 1943.

A case of oligodendroglioma of the left frontal lobe is reported. Skiagraphs showed a dense shadow (7×7 cm.) in the left frontal region, attributable to the calcified tumor. —W. J. B.

EAR

Malignant Tumors of the Middle Ear and the Mastoid Process. FIGI, F. A., and HEMPSTEAD, B. E. [Mayo Clinic, Rochester, Minn.] *Arch. Otolaryng.*, **37**:149-168. 1943.

The report is based upon a study of 38 cases in which treatment was attempted and 10 in which the tumors were too advanced in their growth to permit therapy. Tumors of the middle ear and mastoid are divided into the intrinsic type—those arising internally, and the extrinsic—those originating in the surrounding skin, parotid, or pharynx. In the present series of treated tumors 25 were classified in the former group and 13 in the latter. The most common symptoms were pain, discharge, ulceration in the affected part, and diminished hearing; while bleeding, involvement of the facial nerve, and vertigo occurred less frequently. Persistent aural discharge often treated unsuccessfully on a symptomatic basis for long periods was, however, the outstanding complaint. Bone involvement was often disclosed by roentgen examination. The most common histologic types were basal and squamous cell cancers, the former obviously of extrinsic origin, the latter of either extrinsic or intrinsic derivation. Extrinsic adenocarcinoma, intrinsic hemangioendothelioma and fibrosarcoma were also encountered. Treatment consisted of electrosurgical excision followed by the implantation of radium. Twenty patients survived for 2 years or longer.—M. J. E.

Adenocarcinoma Involving the Middle Ear. GRABSCHEID, E. [New York, N. Y.] *Arch. Otolaryng.*, **37**:430-433. 1943.

The tumor was removed following radical mastoidectomy. The patient received postoperative irradiation and appeared tumor-free 4 years later.—M. J. E.

Neurinoma of the Facial Nerve in the Middle Ear and Mastoid; Report of a Case. ROBERTS, G. J. [Univ. of Southern California Sch. of Med., Pomona, Calif.] *Arch. Otolaryng.*, **37**:62-73. 1943.

The patient, aged 50, had a peripheral facial palsy of 30 years duration, associated with aural discharge and deafness for 15 to 20 years. Exposure of the mastoid disclosed a neurinoma filling the cavity. The tumor extended into the middle ear, tubal region, and middle cranial fossa where it was attached to the dura. It was shelled out successfully, but deafness and facial palsy persisted.—M. J. E.

BREAST

Cystosarcoma Phylloides. With a Consideration of Its More Malignant Variant. COOPER, W. G., JR., and ACKERMAN, L. V. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Surg. Gynec. & Obst.*, **77**:279-283. 1943.

Three cases are reported; in one there was metastasis to the axillary lymph nodes. Nine figures show the lesion in gross and microscopic form.—J. G. K.

Carcinoma of the Breast. DAVIS, H. H. [Omaha, Nebr.] *Nebraska M. J.*, **27**:130-132. 1942.

The author points out that the classic attributes of breast carcinoma are features of the later stages of the disease and may be absent in the early stage when recognition might lead to permanent cure. Any single solid lump should

have an immediate biopsy. It is believed that chronic mastitis or a single trauma rarely leads to the development of carcinoma, but papillomas commonly become malignant. If biopsy with frozen sections followed by radical mastectomy is performed in the early stages of the disease, 60 to 75% of the patients are cured, but the percentage is low if axillary lymph node metastasis has already occurred. X-ray therapy alone rarely cures.—E. E. S.

Mammary Cancer in Youth. DE CHOLNOKY, T. [New York Post-Graduate Med. Sch. and Hosp., Columbia Univ., New York, N. Y.] *Surg., Gynec. & Obst.*, **77**:55-60. 1943.

Seventy-three cases of mammary cancer in patients under 30 years of age are reviewed. Five year survivals in patients operated upon were found to be 40.8% and 10 year survivals, 37%, the results being comparable to those obtained in more advanced age groups.—J. G. K.

Extramammary Paget's Disease. PARSONS, L., and LOHLEIN, H. E. [Reno, Nev.] *Arch. Path.*, **36**:424-427. 1943.

Report of a case of extramammary Paget's disease involving the skin and sweat glands of the groin and scrotum, with 2 figures.—J. G. K.

Osteochondrosarcoma of the Breast. SULLIVAN, S. J. [Chicago, Ill.] *Illinois M. J.*, **82**:140-141. 1942.

A case report.—M. E. H.

Female Genital Tract

Hydatidiform Mole and Chorionepithelioma. ALLEN, J. D., and ALLEN, J. D., JR. [Kentucky Baptist Hosp., Louisville, Ky.] *Urol. & Cutan. Rev.*, **47**:22-26. 1943.

This is a concise discussion. The quantitative Ascheim-Zondek test is considered the most important single factor in the diagnosis of these conditions. The reaction in this test is very strongly positive, more so than in normal pregnancy.—V. F. M.

Cancer of the Cervix. The Effect on the Rate of Cure of Increased Roentgen Radiation to the Parametria. HEALY, W. P., and TWOMBLY, G. H. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **49**:519-530. 1943.

Nine hundred and twenty cases of primary carcinoma of the cervix observed at Memorial Hospital from 1932 to 1937 inclusive are reviewed. The peak in age incidence lay between 40 and 55 years. Only 24 women were unmarried, and 6 of these had borne children. Of foreign born patients, Italians showed the highest incidence, 13.7%; negroes formed 8.5% of the group; and Jews only 5%, in spite of the large Jewish population in New York City. Treatment consisted of divided doses of x-ray to a total of 2,000 to 2,400 r (air) to each of 6 pelvic fields, with intracervical and intravaginal radium. The overall 5 year cure rate was 35.4%. This is in contrast to a rate of 28.5% in a comparable group of patients treated in the preceding 5 year period with essentially the same radium exposures, but with single "massive" x-ray doses of about 700 r (air) to each of 4 pelvic ports.—E. H. Q.

Primary Carcinoma of the Vagina, with a Report of Three Cases. JOHNSTON, H. W. [Toronto, Canada] *Canad. M. A. J.*, **47**:252. 1942.

Primary carcinoma of the vagina is very rare and usually fatal. Of the 3 patients reported, 2 were subjected to radium therapy and to surgical resection, respectively, and appear to be cured 4 and 6 years after treatment.—A. C.

The Role of the Gynecologist in the Field of Cancer. Janeway Lecture, 1942. HEALY, W. P. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **49**:1-10. 1943.

This is a general discussion. It is stressed that the gynecologist should be familiar with all the weapons against cancer—surgery, radiation, endocrine therapy—and be prepared to employ them.—E. H. Q.

Gynandroblastoma of the Ovary. MECHLER, E. A., and BLACK, W. C. [Univ. of Colorado Sch. of Med. and Hosps., Denver, Colo.] *Am. J. Path.*, **19**:633-653. 1943.

Report of a case, with detailed review of the literature. The term gynandroblastoma is used to describe a clinical-pathological syndrome. The ovarian tumor has tubules lined with epithelium and interstitial cell groups in common with arrhenoblastoma, but the histological pattern is not constant. The authors suggest that the gynandroblastomas are teratomas.—J. G. K.

Gynecology: Neoplasms of the Ovary. MEIGS, J. V. [Harvard Med. Sch., Boston, Mass.] *New England J. Med.*, **228**:52-60. 1943.

Neoplasms of the ovary are grouped into (1) those arising from the celomic epithelium covering the ovary, (2) from the primitive mesenchymal cells, (3) from tissues adjacent embryologically, (4) from fertilized ova, and (5) metastatic tumors. The members of each class are discussed briefly with reference to pathology, clinical features, and treatment. The role of the pathologist, surgeon, and roentgenologist in the management of these cases is outlined, and the necessity of cooperative effort is emphasized. The endocrinology of the tumors also is discussed. The bibliography includes about 100 references to the literature. —C. W.

Male Genital Tract

New Developments in the Treatment of Prostatic Carcinoma. CURTIS, M. S. *U. S. Nav. M. Bull.*, **41**:1022-1035. 1943.

A review of the literature on hormonal therapy in prostatic carcinoma and a report on 27 cases.—C. W.

Stilboestrol for Prostatic Enlargement. DODDS, E. C., and WALKER, K. CORRESPONDENCE. *Brit. M. J.*, **2**:436. 1943.

The authors have observed 8 cases of carcinoma of the prostate, which have been rendered completely symptom-free by stilbestrol treatment. Side effects were slight and often entirely absent.—E. L. K.

Treatment of Prostatic Carcinoma by Oestradiol and Diethylstilboestrol. DUNCAN, G. H. *Brit. M. J.*, **2**:137. 1943.

Three cases of carcinoma of the prostate are described, in which treatment with estradiol benzoate and diethylstilbestrol produced a definite clinical improvement.—A. H.

Mesothelioma of the Epididymis and Tunica Vaginalis. EVANS, N. [Los Angeles County Hosp. and Coll. of Medical Evangelists] *J. Urol.*, **50**:249-254. 1943.

Tumors of the epididymis are rare. The author reports 5 instances of a particular type, which he names mesothelioma. These tumors have not heretofore been recog-

nized as a group but have been described by a variety of names such as adeoma, adenocarcinoma, or lymphangioma. The neoplasm is said to arise from the mesothelial cells of the tunica vaginalis.—V. F. M.

Urinary System—Male and Female

Renal Tumors. Kirk, E. J., and Tollman, J. P. [Univ. of Nebraska, Coll. of Med., Omaha, Nebr.] *Nebraska M. J.*, **27**:170-173. 1942.

Records of 23 cases are reviewed. Renal tumors are said to produce symptoms through pressure, necrosis, hemorrhage, extension, and metastasis. Hematuria was the presenting symptom in 19 cases of this series. Eight patients had had at least sporadic symptoms for over 2 years. The radiographic findings characteristic of renal tumors are listed as "elongation of calyces, encroachment of the pelvis, secondary pyelectasis, displacement of kidney and pelvis, deformity of ureteropelvic junction and upper ureter, enlargement of renal contour, and displacement of adjacent structures."—E. E. S.

Malignant Tumors of the Kidney. Surgical and Prognostic Significance of Tumor Thrombosis of the Renal Vein. McDonald, J. R., and Priestley, J. T. [Mayo Clinic, Rochester, Minn.] *Surg., Gynec. & Obst.*, **77**: 295-306. 1943.

Clinical discussion, with 15 illustrative figures.—J. G. K.

Total Cystectomy for Carcinoma of the Bladder. Priestley, J. T., and Strom, G. W. [Mayo Clinic, Rochester, Minn.] *J. Urol.*, **50**:210-227. 1943.

The authors have employed total cystectomy for the following types of neoplastic growth: (1) very extensive low grade neoplasms; (2) repeatedly recurring low grade neoplasms; (3) tumors with multiple foci of origin suggesting a possibility of further malignant change; and, (4) high grade neoplasms that seem after careful study to be limited to the bladder. The authors' technic is described in detail. Their operative mortality for bilateral ureterosigmoidostomy was 15.7%; for the second operation of cystectomy, 11.5%. Twenty-four of 51 patients who survived cystectomy are alive, but the postoperative interval is too brief to permit an accurate estimate of the ultimate survival rates. Eight patients are alive 1 to 28 years after cystectomy.—V. F. M.

Oral Cavity and Upper Respiratory Tract

Neurilemmoma of the Nasal Septum. Bogdasarian, R. M., and Stout, A. P. [Presbyterian Hosp., and Coll. of Physicians and Surgeons, New York, N. Y.] *Arch. Otolaryng.*, **38**:62-64. 1943.

The tumor was successfully excised from the nasal septum of a patient with a longstanding history of nasal obstruction.—M. J. E.

An Unusual Nasal Tumor. Ellis, B. E. [Indiana Univ., Indianapolis, Ind.] *Arch. Otolaryng.*, **38**:65-68. 1943.

A papillary adenocarcinoma involving one nasal cavity and the ethmoid sinuses was extirpated in two stages from a girl of 12 years. Postoperative irradiation was administered (2,100 r), and the patient appeared symptom-free 2 years later.—M. J. E.

Unusual Neoplasm of the Antrum. Harbert, F. *U. S. Nav. M. Bull.*, **41**:1405-1408. 1943.

This is a report of an undifferentiated carcinoma of the maxillary sinus with metastatic extension in the cancellous tissue of the vertebrae without destruction of the bony trabeculae thus making x-ray diagnosis impossible.—C. W.

Criteria for the Selection of Treatment of Cancer of the Larynx. Jackson, C. L., and Blady, J. V. [Philadelphia, Pa.] *Arch. Otolaryng.*, **37**:672-679. 1943.

While excellent results following the treatment of cancer of the larynx by laryngofissure, laryngectomy, and irradiation have been reported, the authors attempt to establish criteria for a choice of each method. Laryngofissure is indicated for lesions occupying a vocal cord, or even for growths reaching the anterior commissure or the opposite cord. Tumors that impair the mobility of the larynx by extension to the posterior extremity of the cord, and those that extend subglottically or invade cartilage, require laryngectomy. Those growing more widely in the cervical region, smaller tumors in individuals whose general health contraindicates surgery, and neoplasms extending to the posterior end of the cord without impairing mobility are best suited for protracted roentgen therapy and implantation of radon.—M. J. E.

Lymphoepithelioma of the Nasopharynx: Report of a Case. Persky, A. H. [Mt. Sinai Hosp., Philadelphia, Pa.] *Arch. Otolaryng.*, **37**:813-818. 1943.

A case report.—M. J. E.

Fibrosarcoma of the Larynx in an Infant. Rigby, R. G., and Holinger, P. H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Otolaryng.*, **37**:425-429. 1943.

An infant with persistent respiratory distress since birth died in its fifth month. Bronchoscopy, dilatation of the larynx, and tracheotomy had been employed as palliative measures. Autopsy disclosed a fibrosarcoma of the wall of the larynx, which, by bulging interiorly, almost obstructed the lumen.—M. J. E.

The Diagnosis of Cervical Metastasis from Squamous Carcinoma of the Mouth and Throat. Whitcomb, C. A. [Jeanes Hosp., Philadelphia, Pa.] *Am. J. Roentgenol.*, **50**:219-229. 1943.

Metastasis from squamous carcinoma about the mouth usually appears as a gradual enlargement of a single cervical node whose location, determined by the site of the primary cancer, is predictable. Clinical diagnosis of metastasis is difficult; biopsy is essential. Small biopsy specimens are adequate for diagnosis, but block dissection gives an opportunity to study the complete histopathological picture.—E. H. Q.

Sarcoma of the Tonsil: Impressions Made by Seven Cases. Whitcomb, C. A. [Jeanes Hosp., Philadelphia, Pa.] *Arch. Otolaryng.*, **38**:1-9. 1943.

Six of the 7 patients under discussion had metastases in the cervical region at the time the diagnosis was established and treatment begun. Therapy given prior to the realization of the true nature of the condition, consisted of the palliative local measures commonly employed for minor ailments of the throat. Roentgen and radium therapy were administered to all patients, but despite temporary amelioration none survived more than 3 years.—M. J. E.

Chondrosarcoma of the Nasopharynx Simulating Juvenile Angiofibroma. Wirth, J. E., and Shimkin, M. B. [Tumor Clinic, Marine Hosp., Baltimore, Md.] *Arch. Path.*, **36**:83-88. 1943.

A detailed report is given of a case of chondrosarcoma of the nasopharynx in a boy 16 years of age. The tumor proved radioresistant; it invaded the cranial cavity and metastasized to the lungs. Seven figures are included.—J. G. K.

Intrathoracic Tumors—Lungs—Pleura

The Diagnosis and Treatment of Primary Intrathoracic Tumors. Dolley, F. S., and Brewer, L. A., III. [Los Angeles, Calif.] *J. A. M. A.*, **121**:1130-1136. 1943.

A review of the clinical signs and symptoms, methods of diagnosis, treatment, and prognosis of primary intrathoracic tumors. Tumors of the lung, mediastinum, superior sulcus, and thoracic cage are discussed. The authors recommend immediate surgical exploration if the diagnosis of an intrathoracic neoplasm has been made by x-ray examination or bronchoscopy. Operation is not indicated in the presence of metastases or when the lesion is known to be a lymphosarcoma.—G. H. H.

Chronic Empyema Due to Dermoid Tumors of the Mediastinum. Dorsey, J. M. [The Presbyterian Hosp., Chicago, Ill.] *Surgery*, **13**:755-761. 1943.

Dermoid tumors of the mediastinum should be considered in the differential diagnosis of chronic empyema. Reports of 2 cases are included in the paper.—W. J. B.

Primary Bronchial Carcinoma at the Age of 4 Years and 4 Months. Field, C. E., and Quilliam, J. P. [University College Hosp., London, England] *Brit. M. J.*, **1**: 691-693. 1943.

A description of an anaplastic carcinoma of the bronchus with numerous metastases.—E. L. K.

The Co-Incidence of Primary Carcinoma of the Lungs and Pulmonary Asbestosis. Homburger, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Am. J. Path.*, **19**:797-807. 1943.

Forty-five cases of pulmonary carcinoma were encountered in 4,137 autopsies during 20 years. Asbestosis was diagnosed 8 times, silicosis 17 times. Pulmonary carcinoma was found in 4 of the 8 instances of asbetsosis and twice in the 17 silicotic cases. Sixteen previously reported cases are cited in which pulmonary asbestosis and carcinoma were associated.—J. G. K.

Gastrointestinal Tract

Treatment of Cancer of the Bowel. Bisgard, J. D. [Omaha, Nebr.] *Nebraska M. J.*, **27**:137-139. 1942.

The mortality from carcinoma of the colon and rectum is recorded as about 30,000 deaths per year. Many cases remain unrecognized because the physician fails to make digital examination of the rectum. Tumors of the left half of the colon more commonly cause obstruction with resulting pain, distention, and vomiting. Those in the right half are more often associated with severe anemia. Recognition and eradication of benign tumors is urged. When resection of a malignant tumor is contemplated either for curative or palliative effect, preoperative reduction of distention and correction of anemia and malnutrition are considered no less important than skill in performing the operation. Preoperative vaccination of the peritoneum has not proved of benefit in preventing peritonitis.—E. E. S.

A Plea for the Earlier Diagnosis of Rectal Cancer. An Analysis of 108 Clinic Patients. Braund, R. R., and Binkley, G. E. [Memorial Hosp., New York, N. Y.] *N. Y. State J. Med.*, **42**:33-37. 1942.

The treatment of cancer of the rectum has improved considerably during the past 25 years. In 1925, the operability in the larger private clinics did not exceed 30%, with an immediate mortality of more than 20%. In 1940, the operability for rectal cancer had reached 60% with an operative mortality of less than 10%. More encouraging has been the corresponding increase in the number of 5-year survivals from 26% to 60% during the same period. Unfortunately, no parallel progress in early diagnosis can be recorded.

In the free clinic of the Memorial Hospital so many new patients with far advanced and incurable rectal cancer were seen that a group of 108 cases were investigated in order to uncover the factors responsible for the delay before treatment was sought at the clinic. One hundred of these patients had seen a physician prior to admission. Seventy percent of the 108 patients remained untreated for an average of 19 months after the onset of symptoms, and one-half of this delay was due to the failure of physicians to make the correct diagnosis. Of the 100 referred patients 20 had not received a rectal examination, their treatment having been based on their symptoms. Thirty-seven had received a digital examination which should have been sufficient to make a tentative diagnosis had the examination been properly performed. In this group, 90% of the rectal cancers were within reach of the examining finger. These findings emphasize again the heavy responsibility that rests on the practicing physician in the early diagnosis, and ultimate successful treatment, of cancer of the rectum. It is the duty of every physician to suspect rectal cancer in any patient who presents himself with complaints referable to any part of the gastrointestinal tract, and a thorough rectal examination should be made. Among early symptoms are blood in the stool, constipation (sudden or gradual), flatus, and frequent bowel movements. More typical symptoms, usually described in the textbooks, are invariably associated with advanced, frequently inoperable, lesions.—A. C.

Total Gastrectomy for Cancer. de Amesti, F. [Univ. of Santiago, Santiago, Chile] *Ann. Surg.*, **117**:183-190. 1943.

Eight cases of gastric carcinoma and 1 of gastric sarcoma are reported. Metastases from the gastric cancers were present, but none were found in the patient with lymphosarcoma. Total gastrectomy was done only when at least the greater part of the stomach was involved without extension to other organs or distant metastases and when mobility of stomach and lower esophagus was adequate. In operating, a left paramedian incision was used with jejunal anastomosis to the esophagus and enteroenterostomy after the severing of the stomach and inversion of the duodenal stump. Splenectomy was performed in 2 individuals. Four patients died postoperatively, the diagnosis being peritonitis in 2, bronchopneumonia in

1, and acute anemia in 1. Death occurred within 11 months in all but 1 patient who was living 13 months after operation.—W. J. B.

Primary Infrapapillary Adenocarcinoma of the Duodenum. Felsen, J., and Wolarsky, W. [Bronx Hosp., New York, N. Y.] *Arch. Path.*, **36**:428-431. 1943.

A case report with 3 figures.—J. G. K.

Surgical Principles Involved in Management of Carcinoma of the Colon. Hunt, V. C. [Los Angeles, Calif.] *Northwest Med.*, **41**:269-272. 1942.

In determining whether or not a tumor is operable, the degree of fixation of the growth should be estimated by palpation and by visualization with the fluoroscope. The possible presence of metastases should also be investigated. The prognosis in lesions of the right half of the colon is considerably better than in those of the left half. The need of preoperative treatment for malnutrition, dehydration, anemia, and obstruction justifies a postponement of operation. A generally poor condition of the patient requires a multiple stage operation regardless of the site of the tumor. Carcinoma in the right half of the colon is more often amenable to one stage resection. When the tumor is in the transverse colon, splenic flexure, descending, or sigmoid colon two operations are usually required. In resecting a carcinoma of the rectosigmoid colon, the advisability of a permanent colostomy must be considered in each case.—E. E. S.

Bone and Bone Marrow

Eosinophilic Granuloma of the Tibia. Case Report. Horwitz, T. [Philadelphia, Pa.] *Am. J. Roentgenol.*, **50**:355-357. 1943.

An instance of eosinophilic granuloma involving the tibia of a 12 year old male is reported. No other bones gave roentgenographic evidence of the disease. The differential white blood cell count revealed a mild eosinophilia. Attention is directed to the specific histopathologic features of this lesion that are unlike those of any classified disease of bone.—E. H. Q.

Fibrosarcoma of the Skull in Paget's Disease. Kirshbaum, J. D. [Sharpsville, Pa.] *Arch. Path.*, **36**:74-79. 1943.

Case report.—J. G. K.

Chondrosarcoma of Bone. Lichtenstein, L., and Jaffe, H. L. [Hospital for Joint Diseases, New York, N. Y.] *Am. J. Path.*, **19**:553-589. 1943.

The authors regard chondrosarcoma as a lesion distinct from osteogenic sarcoma of bone. It develops from cartilage, whereas the latter issues from more primitive bone-forming mesenchyme. In comparison with osteogenic sarcoma, chondrosarcoma is less common, appears at a later age on the average, runs a much slower course, and, especially when excised early, has a much better prognosis. Local trauma does not seem to be a factor in the malignant transformation of the benign growths (enchondroma and osteochondroma) from which chondrosarcomas evolve. Cartilage tumors are regarded as malignant when they present (1) many cells with plump nuclei, (2) more than an occasional cell with two such nuclei, and especially (3) any giant cartilage cells with large single or multiple nuclei or with clumps of chromatin. Twenty-six figures illustrate the lesion.—J. G. K.

Osteogenic Sarcoma. I. A Modified Nomenclature and a Review of 118 Five Year Cures. Macdonald, I., and Budd, J. W. [Univ. of Southern California Sch. of Med., Los Angeles, Calif.] *Surg., Gynec. & Obst.*, **77**:413-421. 1943.

General discussion, with 8 figures.—J. G. K.

Multiple Myeloma with Laryngeal Involvement. Pearson, B., Stark, E., and Kepl, M. [Tulane Univ. Sch. of Med., New Orleans, La.] *Arch. Path.*, **36**:321-323. 1943.

Case report.—J. G. K.

A Case of Multiple Myeloma with Liver Infiltration and a Low Prothrombin Purpura. Schindler, J. A. [Monroe, Wis.] *Ann. Int. Med.*, **19**:140-143. 1943.

Case report.—J. G. K.

A Discussion of the Pathology and Histogenesis of Ewing's Tumor of Bone Marrow. Stout, A. P. [Coll. of Physicians and Surgeons, New York, N. Y.] *Am. J. Roentgenol.*, **50**:334-342. 1943.

The principal features of the pathology of Ewing's tumor are presented. The hypothesis of Ewing that the tumor is derived from vascular or perivascular endothelium, and that of Oberling that it is derived from young reticular cells are discussed. The author favors the latter theory, but believes that a definite decision is not possible with present knowledge.—E. H. Q.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Aleukæmic Lymphatic Leukæmia, or Lympho-Sarcoma. Auld, J. W. [Calgary, Canada] *Canad. M. A. J.*, **47**:563-564. 1942.

A report of a case of lymphosarcoma that appeared as multiple lesions on the scalp and the face soon after an automobile accident that had caused head injury.—A. C.

Lesions of the Mouth in Myeloid Leukemia. Beinfield, H. H. [Brooklyn, N. Y.] *Arch. Otolaryng.*, **38**:69-70. 1943.

The lesion described was an ulcerated enlargement in the pharyngeobuccal region, which resembled a peritonsillar abscess. Its leukemic nature was first suspected after examination of the blood.—M. J. E.

Local Myelopoiesis in Myeloid Leukemia. Schiller, W. [Cook County Hosp., Chicago, Ill.] *Am. J. Path.*, **19**:809-837. 1943.

Two cases of myeloid leukemia are presented in which the endothelial (Kupffer) cells of the liver were transformed *in situ* into monocytes and eosinophils, respectively, as demonstrated by the presence in them of oxidase-positive and eosinophile granules. The findings, in addition to those observed by Jaffé in a similar case previously reported, are held to prove that extramedullary myelopoiesis may occur in myeloid leukemia. Nine figures illustrate the transformations.—J. G. K.

Abdominal Lymphosarcoma. Tollman, J. P., and Eason, E. [Univ. of Nebraska, Coll. of Med. Omaha, Nebr.] *Nebraska M. J.*, **27**:103. 1942.

A case report.—E. E. S.

Pancreas

The Mechanism of Jaundice in Cancer of the Pancreas. Kaplan, N., and Angrist, A. [Queens Gen. Hosp., Jamaica, N. Y.] *Surg., Gynec. & Obst.*, **77**:199-204. 1943.

Of 39 patients with cancer of the pancreas 19 had jaundice. In all jaundiced patients there was carcinomatous invasion of some part of the biliary tract; obstruction by compression alone was not encountered. Four photomicrographs and 7 drawings illustrate the mechanisms of obstruction.—J. G. K.

Islet Cell Adenoma of the Pancreas Associated with Bilateral Urinary Calculi. Spangler, P. E. *U. S. Nav. Md. Bull.* **41**:1087-1097. 1943.

Report of a patient who had a severe metabolic disturbance manifested by two attacks of hemiplegia during convalescence from a kidney operation. These attacks were relieved by dextrose. The disturbance of sugar metabolism was corrected by removal of the adenomas from the pancreas.—C. W.

Pituitary

Eosinophilic Pituitary Adenoma. Haynes, G. O. [Veterans Administration, Washington, D. C.] *M. Bull. Vet. Admin.*, **20**:80. 1943.

Report of a case that was notable for the absence of visual changes and the possible therapeutic benefit of x-ray treatment.—M. E. H.

Thymus

Thymic Tumor in Myasthenia Gravis. A Case Report. Haynes, E. [Madison, Wis.] *Wisconsin M. J.*, **42**: 932-933. 1943.

A case report.—M. E. H.

Thyroid

Hemangioendothelial Sarcoma of Thyroid Gland. Bosse, M. D. [Western Pennsylvania Hosp. Inst. of Path., Pittsburgh, Pa.] *Arch. Path.*, **36**:316-318. 1943.

Case report.—J. G. K.

Miscellaneous

Retroperitoneal Dermoid. Bottone, J. J. [King's County Hosp., Brooklyn, N. Y.] *Urol. & Cutan. Rev.*, **47**:27-28. 1943.

A 52 year old male was found to have a retroperitoneal dermoid cyst (proved by biopsy). Perirenal air insufflation was considered most helpful in diagnosis.—V. F. M.

Carcinoma Which Simulates Sarcoma. A Study of 110 Specimens from Various Sites. Brooks, S. McL. [Manchester, N. H.] *Arch. Path.*, **36**:144-157. 1943.

A review of the literature and general discussion with notes on 12 cases and 6 figures.—J. G. K.

Extramedullary Plasma Cell Tumors As Observed in Various Locations. Hellwig, C. A. [St. Francis Hosp., and the Sedgwick County Tumor Clinic, Wichita, Kans.] *Arch. Path.*, **36**:95-111. 1943.

A general review.—J. G. K.

Classification of Epithelial Cancers Based upon Site of Origin. Merritt, E. A., and Caulk, R. M. [Garfield Memorial Hosp., and Warwick Memorial Clinic, Washington, D. C.] *Am. J. Roentgenol.*, **49**:99-103. 1943.

More than 2,000 cases of cancer treated in a single radiological clinic are classified according to site of origin, instead of pathologic or microscopic diagnoses. It is stated that a cross index file based on this classification makes cases readily accessible for continued observation.—E. H. Q.

So-Called Retroperitoneal Lipoma. Report of Seven Cases. Regan, J. S., Sanes, S., and MacCallum, J. D. [Buffalo Gen. Hosp., and Univ. of Buffalo Med. Sch., Buffalo, N. Y.] *Ann. Surg.*, **117**:110-117. 1943.

In the cases reported, there were 2 males and 5 females. The microscopic structure of 3 tumors was sarcomatous. It was possible to remove the growth in 4 cases, however 1 of these patients succumbed postoperatively. References are given to other reports in the literature of the last 10 years.—W. J. B.

Newer Aspects of Cancer Research. Caldwell Lecture, 1942. Rhoads, C. P. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **49**:289-298. 1943.

The author discusses the experimental and clinical data bearing on the role of the sex hormones in the production of cancer of the genitalia. He concludes that no definite etiologic relation can be clearly established in man, but that these hormones do have some influence on acceleration and retardation of the rate of growth of mammary and prostatic cancer.—E. H. Q.

Cases from Tumor Clinic. Trueblood, D. V. [Seattle, Wash.] *Northwest Med.*, **41**:132. 1942.

Brief reports are given on 3 cases of tumor of the jaw, tongue, and breast, respectively.—E. E. S.

Why Should Cancer Cases Be Reported? Keettel, W. C. [Wisconsin State Board of Health, Madison, Wis.] *Wisconsin M. J.*, **42**:790-791. 1943.

Accurate and complete reporting will furnish much needed information and aid in the effectiveness of lay education programs.—M. E. H.

Book Reviews

OBZCHAYA I CHASTNAYA ONKOLOGIA. [GENERAL AND SPECIAL ONCOLOGY.] Edited by A. V. Melnikov. "Medgiz," Narkomzdrav, S.S.S.R., Moscow-Leningrad. Volume 1. 1940. 615 pages, 181 photographs. In Russian. Price, bound, 41 rubles, 50 kopeks.

The first volume of this comprehensive textbook, written by fifteen Soviet authorities, covers the subjects of comparative and experimental oncology.

I. P. Mistchenko contributes two papers to the section on comparative oncology, one on tumors of vertebrate animals and one on tumors of plants. The biology of the cancer cell is covered by A. D. Timofeevsky, and its biochemistry by E. I. Sterkin. The section on malignant tumors in general includes six reports: A. D. Timofeevsky on transplantation of tumors; M. M. Fomenko and S. N. Ledanov on the morphology of transplanted strains of tumors; M. A. Magat on the experimental production of tumors; L. M. Schabad on the morphology and histogenesis of tumors induced by chemical agents; N. D. Iudina on the effect of carcinogenic compounds on the blood and blood-forming organs; and A. D. Timofeevsky on tissue culture of tumors. The relations between tumor and host are discussed in nine papers: R. E. Kavetsky on the etiology and pathogenesis of malignant tumors; M. B. Medvedeva on metabolism in the presence of tumors; A. A. Bogomoletz on cancer and anergy of the mesenchyme; N. N. Sirotinin on allergy and cancer; I. M. Neiman on immunity to malignant tumors; L. M. Shabad on experimental genetics; L. F. Larionov on endocrine glands and cancer; I. P. Mistchenko on diet and cancer; and S. I. Zalkind on mitogenetic radiation and cancer.

On the whole, the textbook is a critical and scholarly presentation of the major aspects of experimental cancer research. The literature is well reviewed and over 3,200 references are cited.

The predominance of the biologic approach in cancer research and the particular emphasis on the effect of tumors upon the organism are characteristic of Soviet experimental oncology, and in these fields Soviet workers are undoubtedly preeminent. One is struck, however, by the practical absence of Soviet work on the genetics of cancer. This deficiency is apparent in the studies on the resistance and susceptibility to transplanted tumors and similar problems in which the genetic factors are not sufficiently appreciated, and in the general use of non-homozygous animals. The existence of mitogenetic rays is not generally accepted in the United States, and the editor of this volume notes that Zalkind ignores the negative reports in his discussion of the subject.

Subsequent volumes are to deal with the general morphology of malignant tumors, the pathology of malignant tumors, general clinical oncology, special clinical oncology, and radiation therapy. This, the first volume of the series, on comparative and experimental oncology, is easily superior to anything of its type available in the English language. In the reviewer's opinion, it would be worth while to translate it *in toto* into English, or, even better, to initiate the creation of a similar text by American and British oncologists.

MICHAEL B. SHIMKIN

TRUDI PERVOVO SYEZDA ONKOLOGOV UKRAINSKOI SOVETSKOI SOTSIALISTICHESKOI RESPUBLIKI. [THE WORKS OF THE FIRST CONVENTION OF ONKOLOGISTS OF THE UKRAINIAN SOVIET SOCIALIST REPUBLIC.] Edited by A. A. Bogomoletz, R. E. Kavetsky, and A. V. Melnikov. "Medgiz," Narkomzdrav, S.S.S.R., Moscow-Leningrad. 1940. 547 pages. In Russian. Price, bound, 45 rubles.

These are the proceedings of the first convention of oncologists of the Ukraine Republic, held in Kiev on May 25–30, 1938. The meeting was attended by 391 delegates, representing 7 of the 11 Republics of the Soviet Union; practically all the workers prominent in experimental oncology in the U.S.S.R. were present. The 93 reports were divided into 10 sessions, with discussions and a summary at the end of each. The subjects of the sessions were: (1) Carcinogenic substances. (2) Biology of the cancer cell; precancer. (3) Resistance and susceptibility to cancer. (4 and 5) Alterations in the cancerous organism. (6) Organization of the anti-cancer campaign. (7) Cancer of the respiratory organs. (8) Biologic therapy and biologic methods of diagnosis. (9) Effectiveness of surgical treatment. (10) Effectiveness of radiation therapy of malignant tumors.

The wide scope of experimental oncology in the Soviet Union, and the desirability of better acquaintance and closer contact with the work of the Soviet oncologists, are apparent from the Resolutions of the Convention (pages 537–539) concerning the reports on experimental oncology:

> "The convention notes that on the subject of substances that elicit malignant tumors the following results were reached: (1) A series of compounds was synthesized and their action investigated. (2) Interesting work is in progress on isolated compounds and their action on cells and on the whole organism. (3) Important specificity of their action as substances that produce malignant tumors was established. (4) A series of various facts was established toward the possibility of carcinogenic compounds arising in the organism and, further, their possible etiologic significance in the origin of spontaneous tumors in animals and man.
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> "The convention considers that further extension of investigations in the problem of carcinogenic substances is essential, including the following: (1) Elucidation of their mechanism of action. (2) Elucidation of the correlation of carcinogenic activity with the chemical structure of the compounds. (3) Further study of the problem of endogenous production of carcinogenic compounds and their etiologic role.
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> "On the problem of the biology of the cancer cell, the following results were reached: (1) It was reaffirmed through new investigations, with the aid of new methods, that blastogenous cells arise from the cells of the organism and are characterized by certain degrees of autonomy. (2) The cancer cell, possessing the property of unrestricted growth, may for a long time exist in a quiescent state, retaining its established properties. (3) The method of tissue culture allows the discovery that malignant cells of man retain to certain limits the histo-blastomatous properties of the tissue of origin and in certain cases have the ability to differentiate. (4) New facts were established in the important field of study of the chromosome structure of the cancer cell. (5) Data were presented touching certain aspects of the metabolism of tumor tissue (proteosynthesis, acid-base systems).

"It is imperative (1) to continue the study of the biology of the tumor cell, particularly human, in the fields of karyology, metabolism of substances, and physicochemical properties, (2) to extend work on the elucidation of the possibility of isolating the factor of malignancy from mammalian cells.

"On the most important question of precancerous states are being conducted numerous investigations. Side by side of extensive clinical observations on the understanding of precancer, the question was enlightened by morphologic, experimental, biologic, and biochemical points of view. On this were received certain new data on the general and local changes in the organism during the precancer period.

"The question requires further detailed study in close collaboration with the clinic.

"On the question of resistance and disposition to cancer the following results were reached: (1) It was established that the organism possesses resistance to malignant neoplasms; the stimulation of this characteristic of the organism constitutes one of the most important problems in the war against cancer. (2) It was established that active mesenchyme plays an essential role in the protection of the organism to blastomatous growth (a view introduced by Acad. A. A. Bogomoletz 14 years ago). (3) The stimulation of active mesenchyme by doses of specific cytotoxic serum restores the carcinolytic property of serum of patients with cancer, which points toward the possibility of achieving a beneficial effect on the mesenchyme of patients with cancer. (4) Data were presented allowing the suggestion that the increase in immunity to malignant tumors is associated with an increase in oxidizing processes and lowering of glycolytic processes, and, conversely, lowered resistance is associated with depressed tissue respiration and increased glycolysis.

"Essential are (1) further studies on the role of active mesenchyme in the pathogenesis of malignant tumors, (2) further studies on the action of cytotoxic antimesenchymal serum on the organism with the objective of developing methods of prophylaxis of recurrences and metastases and general treatment of cancer, (3) further studies in the question of specific immunity to malignant tumors, on the antigenic properties of the cancer cell, and possibly anti-tumor vaccination, (4) further studies of the bio-physico-chemical nature of the immunizing substance to malignant tumors.

"New experimental results were presented showing the role of the nerve component in the distribution of metastases. Further wide study of this question is essential, as well as of the whole problem of the nervous system in the origin and course of tumors.

"Reports were presented on the subject of cachexia in cancer, from clinical as well as experimental standpoints. Special attention was devoted to the importance of full-value protein and vitamin diet in patients with cancer. Further work on the pathogenesis of cancer cachexia through investigations in metabolism is essential.

"For the guarantee of the possibility of extending the investigations noted above, the convention considers essential: (1) Organization of laboratories of organic synthesis for the production of carcinogenic and anticarcinogenic chemical compounds. (2) organization of laboratories for biologic investigation, on animals, of the possible carcinogenic action of compounds suspected of possessing such properties and having importance in industry or which may be widely used by the general population. (3) The initiation of studies, in special oncological institutes, on the problem of biologic and chemical methods of cancer therapy and the study of methods of biologic diagnosis. (4) It is considered desirable to obtain from the United States or France a series of homozygous strains of mice and other laboratory animals and to assure their maintenance in several central laboratories. (5) To procure strains of tumors that are important for experiments and not available in the U.S.S.R., (6) To initiate the problem of producing new strains of tumors (of dogs, monkeys, and other animals). (7) To develop methods of producing tumors by chemical products in new types of animals."

MICHAEL B. SHIMKIN

DICTIONARY OF BIO-CHEMISTRY. William Marias Malisof, Editor-in-Chief. Philosophical Library, New York, 1943. 579 pages. Price $6.00.

The "Dictionary of Bio-Chemistry," as stated in its preface, was designed for readers of biochemical literature. The book is a cross between an alphabetical glossary and something resembling a condensed encyclopedia. However, whether a book of this type is a mere glossary or an encyclopedia, its value depends entirely upon its degree of accuracy in defining biochemical terms and compounds. This book contains misleading information and so many inaccuracies and poor definitions that, despite some good but brief articles, it cannot be recommended for readers of biochemical literature. To mention only a few of its shortcomings and errors, coenzyme I is said to be a mononucleotide and not differentiated from coenzyme II; lysozyme, which is a protein, is stated to contain no nitrogen; and urea is said to react *in vivo* with glycine to form glycocyamine. Also, the configuration of some of the natural amino acids is sometimes said to be *levo–* and sometimes *dextro–*. Anyone wishing to understand the recent controversy over Kögel's theory on the occurrence of *d*-amino acids in tumors would find this book a hindrance rather than an aid.

DAVID SHEMIN

Correction

The authors of The Metabolism of Normal and Tumor Tissue (3:73-87. 1943) request that the following correction be made. It is made thus tardily because their first letter respecting it was lost in transit.

In Tables III and V the second column, which now reads:

Liver tumor
" "
" "
etc.

should read as it was in their manuscript:

Liver
Tumor
Liver
Tumor
Liver
Tumor
etc.,

the first horizontal line of figures giving the results with liver tissue, the second horizontal line the results with tumor tissue, and so on.